THE
AMERICAN JOURNAL OF MEDICAL TECHNOLOGY

EDITOR-IN-CHIEF

Rose Matthaei
M.T. (ASCP)
Suite 25, Hermann
Professional Bldg.
Houston 25, Texas

EDITORIAL STAFF

ADVISORY EDITORS

Frieda Claassen
M.T. (ASCP)
St. Paul, Minnesota

Mrs. Doris Whitney
M.T. (ASCP)
Roselle, Illinois

ASSOCIATE EDITORS

Evelyn Ballou
M.T. (ASCP)
Washington, D.C.

Nellie Mae Bering
M.T. (ASCP)
Washington, D. C.

Mary Benedict Clark
M.T. (ASCP)
Louisville, Kentucky

Elsbeth Ellis
M.T. (ASCP)
Miami Beach, Florida

Esther Freier
M.T. (ASCP)
Minneapolis 11, Minnesota

Leanor D. Haley
Ph.D. M.T. (ASCP)
New Haven, Connecticut

Mrs. Elsa Kumke
M.T. (ASCP)
Detroit, Michigan

Josephine Pyle
M.T. (ASCP)
Middletown, Conn.

Patricia Sallas
M.T. (ASCP)
New Orleans, La.

**Sister M.
Simeonette Savage**
Spec. Bact. (ASCP)
Chattanooga, Tennessee

Angela Serafini
M.T. (ASCP)
Detroit, Michigan

Genevieve Stout
M.T. (ASCP)
Chamblee, Georgia

The publishers accept no responsibility for opinions expressed by contributors.

CONTENTS

VOLUME TWENTY-TWO NUMBER 6a

DECEMBER

1956

Page

DEDICATION 17

SYMPOSIUM: Practical Laboratory Approaches to Enteric Disease With Special Emphasis on Cold Climate Aspects..... 19

On the Principles of Biochemical and Serological Differentiation of Enterobacteriaceae..... 19
By Dr. F. Kauffmann

Newer Biochemical Methods in the Recognition of Shigellae and Salmonellae..... 28
By P. R. Edwards, Ph.D.; Mary A. Fife and W. H. Ewing, Ph.D.

Experience With the Laboratory Diagnosis of Enteric Diseases in Alaska..... 36
By Harold J. Fournelle, Ph.D.

Experience With the Laboratory Diagnosis of Enteric Disease in Canada..... 44
By W. R. Bailey, Ph.D.

Symposium Discussion 52
By E. T. Bynoe, Ph.D.

AMONG THE NEW BOOKS..... 55

SYMPOSIUM: Electrophoresis..... 56

Electrophoresis in the Clinical Laboratory 56
By Antoinette M. Caldarella, B.s., M. T. (ASCP)

Electrophoretic Studies of Sickle Cell Anemia... 61
By Eleanor E. Stephenson

Paper Electrophoresis—General Principles..... 65
By Kathryn Catalano

An Electrically Heated and Thermo-Statically Controlled Oven for Drying Electrophoresis Paper Strips 69
By Charles D. Brown

AWARDS 72

The American Journal of Medical Technology is owned by The American Society of Medical Technologists. It is published bi-monthly. The volume begins with the January issue.

Public Relations Counsel, Glassner & Associates, Chicago, Illinois.

American Society of Medical Technologists

BOARD OF DIRECTORS

President: C. Patton Steele, P. O. Box 1020, Bismarck, North Dakota
President-elect: Leanor Haley, Ph.D., 310 Cedar Street, New Haven, Connecticut
Treasurer: Mrs. Kathryn Dean, 1312 Silverthorne Road, Baltimore 12, Maryland
Secretary: Ellen Anderson, Pathology Dept., Med. School, Univ. of North Carolina, Chapel Hill, North Carolina
Ruth Heinemann, 3436 - 47th Avenue, South, Minneapolis, Minnesota
Sister Mary Simeonette (Savage), Clin. Lab., Memorial Hosp., Chattanooga, Tenn.
Mrs. Elsa Kumke, 7726 East Jefferson, Detroit 14, Michigan
Anna Bell Ham, 1190 South Alhambra Circle, Coral Gables, Florida
Jane Taylor, 357 N. Chesterfield Road, Apt. 11, Columbus 9, Ohio
Patricia Salias, Charity Hospital, Dept. of Pathology, New Orleans, Louisiana
Barbara Isbell, 3725 Oleander Drive, San Diego 6, California

ADVISORY COUNCIL OFFICERS

Chmn.: Mary Elizabeth Dempsey, 1704 Pinehurst Avenue, St. Paul 15, Minnesota
Vice Chairman: Frank Pauls, P1242 F St., Anchorage, Alaska
Recording Secretary: Mrs. Jeanne L. Shlafman, 1150 So. Main St., Dayton 9, Ohio
Asst. Recording Secretary: Sister Ann Marie Skinner, St. Johns Hospital, 307 So. Euclid, St. Louis 10, Mo.
Corresponding Secretary: Lydia E. Brownhill, 132 Derby Ave., New Haven, Conn.

EXECUTIVE SECRETARY

Rose Matthaei, Suite 25, Hermann Professional Building, Houston 25, Texas

COMMITTEE CHAIRMEN

Finance: Thelma Woods, 819 Centinela Avenue, Santa Monica, California
Public Relations: Elizabeth O'Connor, 2203 Ridge Avenue, Evanston, Illinois
Education: Mrs. Jacqueline Bahrenburg, 2708 West Broadway, Spokane, Wash.
Standards & Studies: Thelma Wilson, 1205 Johnstown Rd., Apt. 2, Beckley, W. Va.
Membership: James L. Cunningham, Box #268, Port Lavaca, Texas
Legislation: Sarah Allene Wise, 1776 Vine Street, Denver 6, Colorado
Constitution & By-Laws: Marian Anderson, 1765 Laurel Avenue, St. Paul 4, Minnesota
Recruitment: Mrs. Audrey Murphy Wines, 5444 Queens, St. Louis, Missouri
Research: Henrietta Lyle, Washington County Hospital, Hagerstown, Maryland
Nominations: Warren Brown, Good Samaritan Hospital, Puyallup, Washington
Civil Service-Armed Services: Ruth Heinemann, 3436 - 47th Avenue, South, Minneapolis 6, Minnesota
Insurance: Mrs. Elsa Kumke, 7726 East Jefferson, Detroit 14, Michigan
Board of Schools: Mrs. Hazel Current Rogers, 918 - 17th Street, Santa Monica, California
Standing Rules: Barbara Isbell, 3725 Oleander Dr., San Diego, Calif.
Certification Study: Frances Anderson, Lab., Fairview Hosp., Minneapolis, Minn.
Scholarship and Loan Fund: Mrs. Catherine Milos, 631 Ridgewood Rd., Oradell, New Jersey

AFFILIATED SOCIETY PRESIDENTS

Emily McAdory, 220 Deadrick Street, Bessemer, Alabama
Mrs. Betty Victors, 234 E. 15th, Anchorage, Alaska
Mary B. Fennemore, 321 W. Almeria Rd., Phoenix, Arizona
Rose Marie Winkler, 2805 North Taylor Street, Little Rock, Arkansas
Mr. Howard Mackey, 770 Cork St., San Fernando, California
Allyne E. Lawless, 285 South Osceola Street, Denver, Colorado
Lydia Brownhill, Whitney Hall, U. of Conn., Storrs, Connecticut
Loretta Mearns, 2300 Ridgeway, Wilmington, Delaware
Nellie Mae Bering, 1332 - 31st St., N.W., Washington 7, D. C.
William Blum, 4202 San Luis, Tampa, Florida
Marlene Burgess, 282 Candler Street, N.E., Atlanta 7, Georgia
Mrs. Elaine Uyehara, c/o St. Francis Hospital, Honolulu, T. Hawaii
Thomas G. Powell, 110 East Jefferson Street, Boise, Idaho
Mrs. Aileen Burge Wright, 328 Sandusky, Jacksonville, Illinois
Elizabeth Pruden, 2725 Maumee, Fort Wayne, Indiana
Dorothy Becker, 1700 W. 7th, Des Moines, Iowa
Marjorie J. Humphreys, Grace Hospital, Hutchinson, Kansas
Jack Phillips, Sunset Heights, Winchester, Kentucky
Clarilynn Philley, 1748 Stuart Street, Baton Rouge, Louisiana
Edward O. Hooghkirk, Westbrook Junior College, Portland 5, Maine
Elaine Silvers, 3217 Sequoia Ave., Baltimore 15, Maryland
Mrs. Albertine Trudel, P. O. Box 23, Montague, Massachusetts
Elmer C. Sanborn, RR 2, Hastings, Michigan
Mary E. Dempsey, 1704 Pinehurst, St. Paul 15, Minnesota
Mrs. Madeline M. Stockdill, 434 Ridgeway, Jackson, Mississippi
Sr. Ann Marie Skinner, St. John's Hospital, 307 S. Euclid, St. Louis, Missouri
Bynum McNeill Jackson, 1344 Crawford Drive, Billings, Montana
Mrs. Helen Pavich, 3527 Walnut, Omaha, Nebraska
Joan R. Veazey, 34 Dolloff Street, Laconia, New Hampshire
Ruth Burdick, 208 Kenilworth Road, Ridgewood, New Jersey
Betty Jean Heim, Espanola Hospital, Espanola, New Mexico
Mary B. Cooper, 335 Kensington Road, Syracuse 10, New York
Helen Chappell, Albemarle Hospital, Elizabeth City, North Carolina
Mrs. Jean Saumur, Dept. of Pathology, U.N.D., Grand Forks, North Dakota
Mrs. Jeanne L. Schlafman, 1150 South Main St., Dayton 9, Ohio
Norma J. Wenstrom, 1213 South Wheeling, Tulsa, Oklahoma
Lenna D. Loyd, 2421 N.W. Johnson Street, Portland 10, Oregon
Andrew Notartomas, 1516 Lafayette Street, Scranton, Pennsylvania
Charlotte H. Hutchison, 823½ Milton Avenue, Rock Hill, South Carolina
Sister Mary Scholastica Lemmer, McKennan Hospital, Sioux Falls, South Dakota
Sara Elizabeth Williams, P. O. Box 162, Lewisburg, Tennessee
Mrs. Dorothy Temple, 430 Thelma Drive, San Antonio, Texas
Virginia Kreutzer, 3014 East 2965 South, Salt Lake City, Utah
Jeredith P. Lamphere, 76 North Union Street, Burlington, Vermont
Mrs. Catherine T. Rowe, 119 Quinlan Street, Lynchburg, Virginia
Bernice Levitsky, 216-10th Ave, North, Seattle, Washington
Clementa R. Proudfoot, Meyers Clinic Hospital Lab., Philippi, West Virginia
Sister Mary William Saller, St. Agnes Hospital, Fond du Lac, Wisconsin
Margaret Ellen Smith, Nurses' Home, Memorial Hospital, Cheyenne, Wyoming
Mrs. Annette F. Fields, P. O. Box 502, Balboa, Canal Zone

The AMERICAN JOURNAL of MEDICAL TECHNOLOGY

VOLUME 22 DECEMBER, 1956 NUMBER 6a

DEDICATION

To Lucille Wallace—we dedicate this special symposium issue of the JOURNAL. Her selfless dreams, her courage, her faith and loyalty to medical technology and to ASMT, will continue to inspire all who knew and loved her.

She truly served with ever increasing strength and humility, and with all the pride and integrity of her profession.

Ida Lucille Brown Wallace died of cancer on October 17, 1956.

About a year and a half before her death, Lucille was employed in the Arctic Research Laboratory in Anchorage, Alaska. One day, thinking of A.S.M.T., as usual, she commented to Dr. Fournelle, her director, that an international note could be added to the program of the First North American Conference of Medical Laboratory Technologists if we could have a world-famous authority like Dr. F. Kauffmann, of Copenhagen, Denmark, to speak to us. Her interest in the cold climate aspects of enteric diseases—ordinarily thought of in terms of more temperate zones—made her feel this subject would be valuable and appropriate. So the Symposium began—and grew—until we were able to present the following foremost authorities in the field —from Denmark, Canada, the United States, and Alaska.

****

The generosity of Lederle Laboratories Division, American Cyanamid Company and North American Cyanamid Limited, made it possible to bring Dr. F. Kauffmann to the conference.

SYMPOSIUM*

Practical Laboratory Approaches to Enteric Disease With Special Emphasis on Cold Climate Aspects.

ON THE PRINCIPLES OF BIOCHEMICAL AND SEROLOGICAL DIFFERENTIATION OF ENTEROBACTERIACEAE

DR. F. KAUFFMANN
Statens Seruminstitut, Copenhagen, S., Denmark

There is general agreement that the *Enterobacteriaceae* are divided by biochemical methods into groups and that these *biochemical groups* are further sub-divided by serological methods into O-*groups* and *sero-types*. It is further accepted to sub-divide these serotypes by biochemical methods into *fermentative types* or *bio-types* and by phages into *phage-types*. The last entity is therefore the *sero-fermentative-phage type*, that is a type which is characterized by its serological, biochemical and phage-behaviour.

Until today there is no agreement whether it is possible to accept the Linnaean system, that means the establishment of families, tribes, genera and species in the sense of Linné.

In a paper on the philosophy of classification Dr. Cowan has posed the question:

"Can we accept the species concept and all that this implies, or must we view our organisms as a huge spectrum composed of gradually merging forms?"

We have answered this question in the negative as a Linnaean system cannot be used and the species concept in a Linnaean sense cannot be accepted. The *Enterobacteriaceae* must be regarded as "a series of interrelated bacterial types which do not lend themselves to sharp division into tribes or into groups" (Enterobacteriaceae Subcommittee, 1954).

Under these circumstances and since nothing is known concerning genetic relationships within the *Enterobacteriaceae*, it is impossible to decide whether a given classification is scientifically right or wrong, but only whether it is more or less practical and expedient. The whole problem must be regarded from the standpoint of practical

* Presented before the First North American Conference of Medical Laboratory Technologists, June 17-21st, 1956, Quebec City, P. Q. Canada.

diagnosis and in the light of our present limited knowledge. Dr. Buchanan has formulated this in the following way:

"There is not much point to the question as to which is right and which is wrong. A better question is, which is the more convenient, better represents relationships, better facilitates diagnosis and proves most useful."

This attitude is reflected in the two Reports of the *Enterobacteriaceae* Subcommittee in Rio de Janeiro and in Rome. In these reports the *Enterobacteriaceae* are sub-divided into biochemical *groups*, not into *tribes*, *genera* and *species*.

Since it is wished to discuss here only the principles of classification, it is neither necessary to describe the single groups individually nor to present a table of their biochemical behaviour. However, since the biochemical tests are the basis of classification, it is desirable to discuss some fundamental facts. It should be stressed that the biochemical differentiation is based on the whole picture of biochemical behaviour and not on the results of one or several tests.

But before we discuss special reactions, I should like to make some general remarks.

In reading and reporting biochemical reactions it is necessary to differentiate between four different results: positive, delayed positive, mutative and negative. The *positive reaction* is designated by a + sign and occurs mostly after 1 day or after 1-2 days.

The *delayed reaction* is designated by (+), that means always positive, but delayed usually after 3-4 days or longer. This reaction is relatively rare and the sign (+) should be used only if the constancy of this delayed reaction is proved in repeated tests with numerous cultures. A typical example of this reaction is the delayed fermentation of trehalose after 3-4 days by *Salmonella paratyphi C*.

If it is not demonstrated that this delayed reaction is regularly delayed and always occurs at about the same time, it should be designated by the letter x.

This sign x means late and irregularly positive or negative, usually *mutative*. For instance this type of fermentation occurs in biochemical type 2 of *Salmonella typhi* with regard to the fermentation of xylose. The differences between the two xylose-types of *S. typhi* are as follows: Type 1 attacks xylose promptly after 1 day, but type 2 ferments xylose by mutation, the fermentation of xylose is usually delayed and irregular or is lacking. It is incorrect to call this reaction a negative one, as positive reactions will be obtained when several tubes are incubated for long periods. When a loopful of a tube which became positive after some days is plated on agar containing xylose, colonies may be isolated which ferment xylose promptly and which will continue to do so. Although this mutative reaction is irregular, this irregular or variable behaviour is very characteristic for the special type. The differential diagnosis between this mutative fermentation x and the delayed positive fermentation

(+) is possible only on the basis of repeated tests of the same culture. A *negative* reaction is designated by the sign — and should be given only when the tubes are observed for at least 14 days.

Within many serotypes there occur several biotypes which may give with the same substance any of the 4 reactions: +, (+), x or —. The sign "v" previously has been used to indicate "various biochemical types," but not to indicate "variable." The term "variable" should be avoided as it has caused misunderstanding and often is taken to mean "irregular." To avoid further misunderstandings, it is proposed to designate "various or different biochemical types" by the letter "d," from "different." If for example one strain of *S. paratyphi B* ferments inositol promptly, but another strain of the same serotype does not attack this substance at all, this behaviour is not "variable," it is constant for both biochemical types which behave differently in this respect. Here we have to do with two distinct types which differ in their reaction on inositol, but are constant in their biochemical behaviour.

When the biochemical reactions are judged correctly by +, (+), x or —, many discrepancies in the literature and misconceptions regarding the instability of biochemical reactions will disappear. It should be stressed that the signs x and d are different, as x means a delayed, usually mutative reaction and d means different biochemical types within a single serotype.

To obtain the whole picture of biochemical behaviour we have at our disposal more than 50 different tests, but are using only 30 tests in routine-diagnosis.

To give you an example of such a biochemical investigation I will show you a table in which the biochemical behaviour of *Salmonella* types is summarized. (Table 1.)

TABLE 1

SALMONELLA

Adonitol	—	Indole	—
Dulcitol	d	H_2S	+
Sorbitol	+	Gelatin	—
L-Arabinose	+	Ammonium glucose	+
Xylose	+	Ammonium citrate	+
Rhamnose	+	KNO_3	+
Maltose	+	Voges-Proskauer	—
Salicin	—	Methylred	+
Inositol	d	Urea	—
Lactose	—	d-tartrate	+
Sucrose	—	l-tartrate	d
Mannitol	++	i-tartrate	d
Glucose	++	sodium-citrate	+
Trehalose	+	Mucate	+
Stern	d	KCN	—

The KCN test (potassium cyanide test) was originally proposed by Braun and is used in the substrate of Moeller. It is a peptone-solution containing KCN at a concentration of 1:13.300. The tubes are stoppered with corks, sterilized by heating in paraffin. In this

medium the growth of *Salmonella, Arizona, Shigella* and *Escherichia* cultures is completely inhibited, but not that of all the other groups. For practical reasons it is important that *Salmonella* and *Shigella* cultures can be excluded, if the KCN test is positive. In our laboratory this test is used regularly in all biochemical investigations.

In a complete biochemical investigation belong the organic acids which play an important role in the differentiation of *Enterobacteriaceae*, not only in the biochemical type-diagnosis, but also in the group-diagnosis. A rapid differential diagnosis betwen *Salmonella* and *Arizona* can be done only by organic acids (after 20 hours incubation), but not by other biochemical tests such as lactose-fermentation and gelatin-liquefaction. Using d-tartrate, citrate and mucate, it is usually possible to differentiate after 20 hours between *Salmonella* and *Arizona*, as *Salmonella* cultures give a positive reaction, but *Arizona* cultures a negative one. Using l- and i-tartrate, *Arizona* cultures can be excluded, if one of these substances or both are fermented after some days. All tested *Arizona* cultures gave a negative reaction in l- and i-tartrate, in contrast to the majority of *Salmonella* cultures. Special *Salmonella* types as *S. paratyphi A* and *S. sendai* which give negative reactions in organic acids can be excluded without difficulties, either by biochemical or serological tests. As some *Salmonella* types give a delayed positive reaction in organic acids, a biochemical differentiation between *Salmonella* and *Arizona* is not always possible, but is in the majority of the cases.

Another example of the usefulness of organic acids in the group-diagnosis is the differentiation between *Shigella* and *Escherichia* types. The organic acids are not fermented by *Shigella* types, with the exception of *Shigella sonnei* which attacks mucate and some other rare *Shigella* cultures which give a delayed and irregularly positive reaction in d-tartrate. *Escherichia* types usually give a delayed positive reaction in d-tartrate and a positive reaction in mucate.

In the biochemical type-diagnosis the organic acids are very useful inside the *Salmonella* and *Klebsiella* groups. The differentiation between *Salmonella paratyphi B* and *Salmonella java* is of special epidemiological importance. *Salmonella paratyphi B* is d-tartrate-negative, while *S. java* is d-tartrate-positive. These two types cannot be differentiated by serological methods, but only by d-tartrate and the slime-wall test.

While cultures of *S. paratyphi B* produces a slime-wall *S. java* cultures are lacking this property.

As this test is not known generally, I should like to describe the simple technique: An agar plate is inoculated in 3 or 4 places with a needle point and the plates incubated overnight at 37° C. and then kept at room temperature for 1-2 days. Usually, after 24 hours at room temperature a slime-wall appears round the colonies. When

the colonies are too close to one another the slime-wall may fail to develop, or may develop only rudimentarily.

A very useful supplement to the biochemical differentiation is the demonstration of *amino acid decarboxylases*, but I will not go into details here as we are not using these tests in routine-diagnosis, but only in special scientific investigations.

Serological Differentiation

To give you a picture of the principles of serological differentiation, I will try to explain the diagnosis of *Salmonella* types. On the basis of different somatic antigens this group is divided into O groups which are sub-divided by different flagellar antigens into serotypes. The somatic antigens are called O antigens, they are thermostable body antigens. The flagellar antigens are called H antigens, they are thermolabile. In *S. typhi* another antigen occurs which is called Vi antigen and which will be discussed later. The antigenic formula of *S. typhi* is 9,12,Vi :d :-. 9,12 are the O antigens, d is the H antigen which is monophasic, that means that the typhoid bacillus occurs only in one phase, in contrast to other types which are diphasic, for example *S. paratyphi B* = 4,5,12 :b :1,2.

4,5,12 are the O antigens and b :1,2 are the H antigens. *b* is the H antigen of phase 1 and 1,2 is the H antigen of phase 2. The first phase should be written: 4,5,12 :b
while the second phase should be written
4,5,12 :1,2
as both phases have the same O antigen.

For simplification we are writing the formula of this type: 4,5,12 :b :1,2. In the majority of *S. paratyphi B* cultures both phases occur simultaneously, but the single bacillus has either the formula 4,5,12 :b or 4,5,12 :1,2. These two phases are not stable, they split off the other phase. From the first phase *b* the second phase 1,2 is split off and from the second phase 1,2 the first phase.

Another type, *Salmonella typhi murium* has the formula 4,5,12 : i :1,2. As you see, the O antigens are identical with *S. paratyphi B* and the second phases too. In reality they are not identical, as the second phase of *S. typhi murium* has a special antigen which was called 3, but omitted from the formula for simplification. For the type-differentiation between *S. paratyphi B* and *S. typhi murium* the first phases are decisive. Therefore, we have to isolate the first phases and to test these in our diagnostic *b* and *i* sera. This can be done by testing several single colonies in slide-agglutination or from Gard-plates, that means swarm-plates which contain 1% agar and the necessary H sera.

To isolate the first phase of *S. paratyphi B* or *S. typhi murium* an H serum of phase 2 is added to the Gard-plates. In this case we are using an H serum of *S. newport* phase 2 as this culture has the formula 6,8 :- :1,2. It occurs only in phase 2 and has not the same O antigens as *S. paratyphi B* or *S. typhi murium*. The H serum used

for these Gard-plates should not contain O or H-antibodies which could inhibit the swarming of *S. paratyphi B or S. typhi murium* phase 1.

If a culture of *S. paratyphi B* is inoculated on the surface of such a swarmplate, the first phase with the antigen *b* will swarm over the whole surface. In the same way, if a culture of *S. typhi murium* is used, the first phase *i*, will swarm. The H antigens *b* or *i* can be diagnosed easily by slide-agglutination.

If we want to isolate the second phase of *S. paratyphi B*, we have to add to the Gard-plate the H serum *b* which will prevent the first phase in swarming. If we add both H sera *b* + *1,2* to the Gard-plate, no swarming will occur. But if the culture is a mixed culture, for example a mixture of *S. paratyphi B* and *S. typhi murium*, the first phase of *S. typhi murium* will swarm.

It is advisable, in all doubtful cases or if new types are involved, to use a Gard-plate containing the H sera of both phases as we can exclude a mixed culture in this way.

Another important rule is to demonstrate the reversion of both phases, if we are testing a new type. I will give you an example: A new type, *S. wandsworth*, has the formula 39:b:1,2, that is a type with the O antigen 39 and the H antigens of *S. paratyphi B*. We isolate both phases *b* and *1,2* in single colonies on agarplates and put one colony of phase 1 = *b* on a Gard-plate containing *b* serum. After incubation for 20 hours the second phase *1,2* has appeared. On the other side, if we put a colony of phase 2 on a Gard-plate + H serum *1,2* the first phase will appear. We have to control whether both these phases contain the same O antigen 39.

I will give you a table which shows the diagnosis of these types in slide-agglutination (Table 2).

TABLE 2

SLIDE-AGGLUTINATION

	Sera				
	O		H		
	4, 5, 12	39	b	1	1, 2
S. paratyphi B phase 1	+	—	x	—	—
S. paratyphi B phase 2	+	—	—	—	x
S. typhi murium phase 1	+	—	—	x	—
S. typhi murium phase 2	+	—	—	—	x
S. wandsworth phase 1	—	+	x	—	—
S. wandsworth phase 2	—	+	—	—	x

The second phase which was in these cases *1,2* is in other types different and is expressed by the formula *1,5* or *1,6* or *1,7*. All these types which have in the second phase the *1* antigenic complex, were called earlier specific-nonspecific phases. But when another phase-variation was discovered which contained in the second phase the e,n . . . complex (*e,n,x*—*e,n,z*$_{15}$ etc.), these names specific-nonspecific

became meaningless, as you will see from the following formulae:

type 1 = b : 1,2
type 2 = i : 1,2
type 3 = b : e,n,x
type 4 = i : e,n,x

If we compare type 1 with type 3 the second phases differentiate these types and should be called "specific", while the 1. phases should be called "nonspecific." The same is valid for type 2 and type 4.

A third sort of phase-variation was described by Edwards & Bruner: Here the l,w antigen occurs in the 2. phase. To have a neutral designation for all these different phases, we have called them phase 1 and phase 2.

Phase 1 contains the antigens a,b,c,d, etc., while phase 2 contains either the l antigenic complex, the e,n-complex or the l,w antigens.

Let us now discuss the Vi antigen which occurs in *S. typhi*, *S. paratyphi C* and in other groups. The Vi serum which is used for the demonstration of this antigen in *S. typhi* is usually prepared by the original *Ballerup* strain as this strain contains the Vi antigen, but not the O and H antigens of *S. typhi*.

If we are using the following 3 sera in slide-agglutination, we get the following results:

O = 9,12	Vi	H = d
—	++	+

As you can see, the living culture of *S. typhi* is not agglutinated by the homologous O serum. This phenomenon is called O-inagglutinability. It is caused by the Vi antigen, if this antigen is well developed. To get an O agglutination, we have to boil the culture. Only if a typhoid culture is lacking the Vi antigen or if this antigen is weakly developed can we get a positive agglutination in O serum with living culture. We have to do here with the so-called V-W variation which can be demonstrated in the following way (Table 3).

TABLE 3
S. TYPHI

	O	Vi	H
V	—	++	+ or —
VW	+	+	+ or —
W	++	—	+ or —
R	—	++ or —	+ or —

I have added to these forms the so-called R form or rough form which is lacking the O antigen, but may contain Vi or H antigen or both these antigens. R forms are often characterized by the morphologically rough appearance of the colonies, but this is not

always the case, especially not, if the Vi antigen is well developed.

Finally I can mention the M forms or mucous forms of *S. paratyphi B* and other types which grow mucoid and are not agglutinated in the living or formalinized state by O or H sera. To demonstrate the O antigen, it is necessary to boil the culture and to test it in tube-agglutination. This M forms contain a special M antigen which is not of diagnostic importance.

After these general remarks on the antigenic structure of *Salmonella* types, I will try to explain to you the principle of the *Kauffmann-White* schema.

The *Salmonella* group is divided into about 30 different O groups and some of these groups are further sub-divided into sub-groups on the basis of different partial antigens. The O groups are further sub-divided into sero-types on the basis of different H antigens. These H antigens are either monophasic or diphasic, in the latter case they are called phase 1 and phase 2. As we can differentiate at least between 12 different H antigens of phase 1 and 8 different H antigens of phase 2, we are working with 96 different combinations of phase 1 and phase 2, that means that we can diagnose 96 different types inside of each O group. To this we have to add the monophasic types which contain the antigens g,m — m,t etc. Therefore we have to reckon with at least 100 different H antigenic combinations inside of each O group. As we have 30 different O groups, it is possible to diagnose 3000 different *Salmonella* types.

Of these 3000 possible types we have found as yet only about 500 types. During the last 30 years the number of serotypes has raised from 20 types which were analyzed by Bruce White to about 500.

The number of 3000 possible *Salmonella* types must be regarded as a minimal number because the *Kauffmann-White Schema* is simplified for practical reasons. If we are using all existing H partial antigens, we can differentiate between at least 25 H antigens of phase 1 and 15 of phase 2. This gives 375 different H antigenic combinations which can occur inside of each O group. As we have 30 different O groups, we can differentiate between more than 10,000 sero-types.

Edwards & Kauffmann have proposed a simplified antigenic scheme in which the number of O groups is reduced to 20 and the number of antigens of phase 1 to 15 and of phase 2 to 2. By this simplified scheme we can diagnose 600 different *Salmonella* types, instead of 3000 by the original *Kauffmann-White Schema*.

I mention this only to show you that the diagnostic antigenic scheme can be simplified or extended, according to the special requirements. The *Kauffmann-White Schema* is situated between the simplified and the complete schema, it has an intermediate position and is a compromise.

The same is valid for all other antigenic schemes, for example the *Escherichia* table. In this group we are working with 140 different O

groups, about 80 different K antigens which are either capsular or envelope antigens and about 40 different H antigens. As these 3 antigens, O, K and H occur in different combinations, it is evident that the number of possible sero-types is very large and goes into the hundred thousands. The combination of only 50 different K antigens with 40 H antigens gives 2000 sero-types inside of each O group. As we have to reckon with at least 200 different O groups, the number of possible sero-types is about 400,000. But with regard to *epidemic infantile enteritis* the situation is not so complicated as we have to do with a restricted number of types which are the most common. This rule is valid for all the groups of *Enterobacteriaceae:* Some few types are very common while a large number of types is very rare.

For the routine-diagnosis of these E-coli types, OK sera are used by which the K antigens can be diagnosed in slide-agglutination. For the O antigen-determination a boiled broth culture must be tested in tube-agglutination and for the H-determination a formalinized broth culture is used in tube-agglutination or the H determination is done by the immobilization-test. In this test, which is very expedient, a drop of a living, motile culture is mixed with a drop of an H serum and observed under the microscope.

Summarizing I should like to stress that the *type-diagnosis* should be done by biochemical and serological methods, while the *group-diagnosis* is done only by biochemical methods. With regard to classification we should remember that it is not the question of what is right and what is wrong, but only what is more convenient and most useful.

NEWER BIOCHEMICAL METHODS IN THE RECOGNITION OF SHIGELLAE AND SALMONELLAE

P. R. EDWARDS, Ph.D., MARY A. FIFE and W. H. EWING, Ph.D.
Communicable Disease Center, Public Health Service,
U. S. Department of Health, Education and Welfare, Atlanta, Georgia

In any laboratory engaged in work on enteric bacteria one of the most vexing problems encountered is the isolation of organisms which, upon preliminary examination, appear to be shigellae or salmonellae, but which fail to react with antisera for those groups. The problem is particularly acute in the clinical laboratories in which diagnoses must be made as rapidly as is consistent with accuracy, and which usually do not have at hand the array of antisera available in the larger public health laboratories. Such a situation will be alleviated by the use of three simple biochemical tests, the results of which are presented here.

It must be said at the outset that in the recognition of enteric pathogens biochemical and serologic tests should receive equal emphasis and, if either of these regimes is slighted, serious errors inevitably result. The occurrence of related antigens in the various groups of enteric bacteria is so pronounced that it is impossible to depend entirely on serologic tests. Perhaps the best illustration of this situation is the close serologic relationships existing between *Shigella* serotypes and members of the *Escherichia* group. Of 40 *Shigella* serotypes listed by Ewing (1953) and by Edwards and Ewing (1955), 35 either were identical with, or were so closely related to, coliform bacteria that agglutination of the latter in *Shigella* antisera could be expected to occur. On the other hand, antisera generally available for the detection of shigellae and salmonellae do not contain agglutinins for certain of the less frequently occurring serotypes, so that a certain number of strains, although they are true members of the *Shigella* and *Salmonella* groups, will not react in antisera of restricted coverage. Thus, careful biochemical tests are necessary to distinguish extraneous forms which react in *Shigella* and *Salmonella* antisera as well as to detect true members of those genera which may fail to react in available antisera.

Included in Table 1 are the biochemical tests most frequently used in the past to differentiate shigellae and salmonellae from other enteric bacteria. Relatively few laboratories employ a greater variety of critical tests to distinguish the organisms. However, even when isolation plates are carefully and judiciously picked and the listed tests are properly applied, a disappointing number of cultures, which upon first observation seem to conform to one or the other of the delineated patterns fail to agglutinate in indicated antisera. Fortunately, simplified tests now are available which serve to differentiate such forms from shigellae and salmonellae and to permit their assignment to groups to which they really belong. These are the lysine

decarboxylase test (ninhydrin test) as modified by Carlquist (1956), the utilization of citrate in the citrate-sulfide medium of Christensen (1949), and the KCN test of Braun (1938) as modified by Moeller (1954).

TABLE 1

Biochemical Tests Usually Employed in the Differentiation of Shigella and Salmonella Cultures

	Shigella	Salmnella
Indol	d	—
Methyl Red	+	+
Voges-Proskauer	—	—
Citrate (Simmons')	—	+[1]
H_2S (from TSI slants)	—	+[2]
Motility	—	+[3]
Glucose	Acid[4]	Acid with gas
Lactose	—[5]	—
Sucrose	—[6]	—
Salicin	—	—
Adonitol	—	—

d = different biochemical types

1. **S. typhi, S. paratyphi A** and a few types rarely encountered fail to grow in Simmons citrate agar.
2. **S. paratyphi A** and a few rare types fail to produce H_2S.
3. **S. pullorum** and **S. gallinarum** are nonmotile. Nonmotile variants of other types appear very rarely.
4. Gas produced by some varieties of **S. flexneri 6**.
5. **S. typhi** and **S. gallinarum** are anaerogenic. Anaerogenic varieties of other types occur very rarely.
6. **Shigella sonnei** ferments lactose and sucrose upon prolonged incubation.

Let us consider first those forms which resemble Shigellae upon triple sugar iron (TSI) agar and which, after overnight incubation of biochemical tests conform to the pattern listed in Table 1, but which do not react in *Shigella* antisera. Such cultures, upon prolonged incubation of biochemical tests, often will ferment lactose, sucrose, adonitol, or salicin or they finally may exhibit some other biochemical property which permits their elimination from the *Shigella* group. However, several days or weeks may pass before this occurs and in the meantime it has not been possible to render a satisfactory report. If the possibilities are examined it quickly becomes apparent that such organisms more likely are aberrant members of the *Escherichia* group than any other group of enteric bacteria. It is known (Christensen, 1949) that *E. coli* types usually utilize citrate to a greater or lesser extent in the presence of readily assimilable sources of carbon and nitrogen, whereas shigellae do not. It likewise is known (Moeller, 1954a, Kauffman and Moeller, 1955) that *E. coli* produces moderately strong lysine decarboxylases, while shigellae either do not produce these enzymes or produce them slowly and in small amount. It is possible to take advantage of these facts to identify aberrant organisms which superficially resemble shigellae but which possess the basic biochemical characteristics of the *Escherichia* group. The ability of the organisms to grow in KCN medium is of little assistance in this instance since neither *Shigella* serotypes nor *E. coli* strains give a positive KCN test.

In tables 2 and 3 are given the results obtained with shigellae and cultures of the Alkalescens-Dispar (A-D) group in the ninhydrin test of Carlquist and in Christensen's citrate medium. Alkalescens-Dispar cultures were selected as convenient representatives of cultures which possess the basic characteristics of the *Escherichia* group but which are not biochemically typical of that group. It will be noted that the shigellae, all known serotypes of which were included in the work, were uniformly negative. The majority of the A-D cultures were positive in both tests but the percentage of positive results (72.2 in the ninhydrin test and 80.0 in the citrate test) are not extremely impressive. However, it should be emphasized that the percentage is lowered by the inclusion in the study of relatively large numbers of A-D cultures of O antigen groups which are encountered very infrequently in diagnostic work. These cultures had been saved from a mass of diagnostic material received over many years. If the A-D groups are proportioned in the tests in accordance with the frequency with which they actually occur in diagnostic material received in this laboratory, results cited in Table 4 are obtained which show the percentage of positive results to be much higher. It is evident that one is very unlikely to meet members of the A-D group which give negative results in both the ninhydrin and Christensen's citrate tests. The citrate medium described by Hajna (1955) also was used in the tests but gave results inferior to those obtained with Christensen's medium.

TABLE 2

Behavior of Shigellae and Alkalescens-Dispar Group in Ninhydrin Test

	No. Tested	No. Positive	No. Negative
Shigella	671	0	671
Alkalescens-Dispar	226	163	63

TABLE 3

Behavior of Shigellae and Alkalescens-Dispar Group in Christensen's Citrate

	No. Tested	No. Positive	No. Negative
Shigella	606	0	606
Alkalescens-Dispar	216	175	41

The objection may be raised that serum for the detection of A-D cultures is available generally and that such cultures do not agglutinate in absorbed *Shigella* grouping sera. It must be emphasized that these cultures were used only as representatives of aberrant coliform bacteria and that, in addition to the A-D cultures, a number of aberrant *Escherichia* cultures of other O antigen groups were examined with similar results. For instance, the cultures described by De Assis (1948) as *Shigella guanabara*, and which now are known as members of *E. coli* O group 112a, 112c, are identical serologically with *Shi-*

gella dysenteriae type 2 (*Shigella ambigua*). A number of Guanabara strains had been stored in the collection of *Shigella dysenteriae* 2 cultures and it was possible to distinguish them easily since 95 per cent were positive in the ninhydrin test and 89 per cent were positive in Christensen's citrate. None was negative in both tests, as were the cultures of *S. dysenteriae* 2. The results obtained with the A-D cultures and with other coliform bacteria on the one hand and with the various *Shigella* serotypes on the other, illustrate the value of the two tests in their differentiation.

TABLE 4

Percentage Incidence of Alkalescens-Dispar O Antigen Groups* and Behavior in Tests

	Percent Incidence	Percent + Ninhydrin	Percent + Citrate
A-D O Group 1	92.4	90.6	95.0
2	6.2	63.0	81.7
3	0.9	62.2	55.9
4	0.2	40.0	100.0
5	0.1	100.0	100.0
6	0.1	50.0	50.0
7	0.1	0.0	28.7
8	0.1	100.0	80.0
Expected Percent Positive, all A-D cultures		88.45	93.82
Expected Percent Positive, **Shigella**		0.0	0.0

*Based on examination of 1024 A-D cultures received for diagnosis.

In the study of those organisms which resemble salmonellae on TSI agar slants, i.e. cultures which blacken the medium and produce gas, there are two situations which delay diagnostic reports. First, there are strains which fail to agglutinate with available *Salmonella* sera or which agglutinate weakly in polyvalent serum but not in O grouping sera and which are not members of the *Salmonella* group. Second, there are those *Salmonella* types which belong to O groups other than those represented in the generally available diagnostic sera and which are agglutinated neither by polyvalent nor O grouping sera. If one examines the biochemical characteristics of the various groups of enteric bacteria it is apparent that the organisms known as *Escherichia freundii* or *Citrobacter freundii*, the Arizona group, and members of the genus *Proteus* are those most likely to resemble salmonellae in TSI agar. The *Proteus* strains generally are eliminated from consideration by positive urease tests although urease negative *Proteus* cultures occasionally are met. The *Escherichia freundii* cultures, including the Bethesda-Ballerup group (Edwards, West and Bruner, 1948; Bruner, Edwards and Hopson, 1949; and West and Edwards, 1954) are much more troublesome since they give negative or delayed positive urease tests and often ferment lactose, sucrose, or salicin only after prolonged incubation or not at all. In the absence of fermentation of one of the above-mentioned substances and of a positive urease test, the biochemical tests listed

in Table 1 do not permit the differentiation of such forms from salmonellae. Under such circumstances, unless one resorts to additional biochemical tests, reports are delayed until a positive fermentation of lactose, sucrose, or salicin can be obtained. In these instances it becomes highly desirable to introduce the use of the ninhydrin test and the KCN test, either of which serves to distinguish *E. freundii* cultures from salmonellae within 24 hours.

The results obtained with the ninhydrin test are given in Table 5. While 4 per cent of the 794 *Salmonella* cultures tested were negative, practically all the negative cultures were *S. paratyphi A*, a readily recognized form. The only other cultures which gave negative tests were *Salmonella typhi-suis*, a type most unlikely to be seen in medical laboratories, and one culture among the large number of *Salmonella typhi murium* strains tested. Included in the *Salmonella* cultures tested were all the known serotypes of the group as well as at least 10 strains of all those types which are encountered frequently in diagnostic work. The Arizona cultures behaved as did the salmonellae but only 2 per cent of the *E. freundii* (Bethesda-Ballerup) cultures were positive.

TABLE 5

Behavior of Salmonella, Arizona and E. freundii Cultures in Ninhydrin Test

	No. Tested	No. Positive	No. Negative
Salmonella	794	762	32[1]
Arizona	417	405	12
E. freundii[2]	507	10	497

1. 30 cultures of **S. paratyphi A** negative.
2. Slow lactose fermenting forms usually referred to as Bethesda-Ballerup Group.

In the KCN test (Table 6) only 1.1 percent of the *Salmonella* cultures were positive while 99 per cent of the *E. freundii* cultures were positive. Again the Arizona cultures behaved in a manner similar to the salmonellae, 92.2 per cent failing to grow in KCN. Of the positive cultures, the majority belonged to one O group of which a disproportionally large number was tested in order to establish their aberrant behavior. There is no apparent explanation for the fact that practically all Arizona O group 21 cultures grow in KCN since otherwise they are typical members of the Arizona group and all were positive in the ninhydrin test.

TABLE 6

Behavior of Salmonella, Arizona and E. freundii Cultures in KCN Test

	No. Tested	No. Positive	No. Negative
Salmonella	900	10	890
Arizona	501	32[1]	462
E. freundii[2]	580	574	6

1. 22 of 23 cultures of Arizona O group 21 positive.
2. Slow lactose fermenting cultures usually referred to as Bethesda-Ballerup Group.

From the results cited above it is apparent that *Salmonella* and Arizona cultures can be distinguished quickly and with certainty from *E. freundii* and related forms by the tests employed. Likewise, any *Proteus* cultures which are urease negative will be eliminated by the KCN test as will Providence cultures (29911 of Stuart, Wheeler and McGann, 1946) which often are forwarded for identification as shigellae or salmonellae.

In conclusion the writers would urge a more thorough biochemical examination of cultures which appear to be members of the *Shigella* and *Salmonella* groups when observed on TSI slants. By this statement it is not meant that reports should be delayed. On the contrary, the use of the tests advocated here actually will result in more prompt reporting of cultures which heretofore have posed diagnostic problems. Objection has been raised (Felsenfeld and Eveland, 1956) that the KCN medium is poisonous and increases hazards in the laboratory. The writers have treated this medium like any other so far as disposal and sterilization are concerned without ill effects. All of the media recommended are easily available and readily prepared.

Summary

It is possible to distinguish shigellae from *Escherichia*-like cultures which may be confused with them by the use of the ninhydrin test and Christensen's citrate-sulfide medium. Likewise, salmonellae can be distiguished from *E. freundii* and related forms by the use of the ninhydrin and KCN tests. The use of these tests in the examination of cultures of enteric bacteria is urged.

REFERENCES

1. Braun, H.: Ueber das aerobe und anaerobe Wachstum der Bakterien unter den Einwirkung von Kaliumoyanid. 2. Mitteilung. Schweiz Z. allgem. Pathol. u. Bakteriol. **1:** 257-272 (1938).
2. Bruner, D. W., Edwards, P. R. and Hopson, A. S.: The Ballerup group of paracolon bacteria. J. Infect. Dis. **85:** 290-295 (1949).
3. Carlquist, P. R.: A biochemical test for separating paracolon groups. J. Bact. **71:** 339-341 (1956).
4. Christensen, W. B.: Hydrogen sulfide production and citrate utilization in the differentiation of the enteric pathogens and the coliform bacteria. Res. Bull. Weld Co. Health Dept., Greeley, Colo. No. 1: 3-16 (1949).
5. DeAssis, A.: Shigella guanabara, tipo serologico destacado do groupo do Geylonensis-dispar. O Hospital. **33:** 505-518 (1948).
6. Edwards, P. R., and Ewing, W. H.: The identification of Enterobacteriaceae. Burgess Publishing Co., Minneapolis (1955).
7. Edwards, P. R., West, M. G., and Bruner, D. W.: Antigenic studies of a group of parocolon bacteria (Bethesda group). J. Bact. **55:** 711-719 (1948).
8. Ewing, W. H.: Serologic relationships between Shigella and coliform bacteria. J. Bact. **66:** 333-340 (1953).
9. Felsenfeld, C., and Eveland, W. C.: Recent trends in laboratory work with Enterobtcteriaceae. Lab. Digest. **19:** No. 12, 14-18 (1956).
10. Hajna, A. A.: Utilization of citrate by gram-negative organisms of the intestinal group together with the formula for a new citrate agar. Pub. Health Lab. **13:** 17-20 (1955).

11. Kauffmann, F., and Moeller, V.: On amino acid decarboxylases of Salmonella types and on the KCN test. Acta Pathol. et microbiol. Scand. **36:** 173-178 (1955).
12. Moeller, V.: Diagnostic use of the Braun KCN test within the Enterobacteriaceae. Acta Pathol. et microbiol. Scand. **34:** 115-126 (1954).
13. Moeller, V.: Distribution of amino acid decarboxylases in Enterobacteriaceae. Acta Pathol. et microbiol. Scand. **35:** 259-277 (1954a).
14. Stuart, C. A., Wheeler, K. M., and McGann, V.: Further studies on one anaerogenic paracolon organism, Type 29911. J. Bact. **52:** 431-438 (1946).
15. West, M. G., and Edwards, P. R.: The Bethseda-Ballerup group of paracolon bacteria. U. S. Department of Health, Education and Welfare, Public Health Monograph No. 22 (1954).

APPENDIX
Biochemical Methods

Ninhydrin Test

Medium

Trypticase (or Casitone)	15 gms.
Dibasic potassium phosphate	2 gms.
Glucose	1 gm.
Distilled water	1000 ml.

Dispense in 5 ml amounts in screw-capped tubes and sterilize at 15 lbs. for 15 minutes. Inoculate from broth or agar culture, incubate at 37 C overnight and add 1 ml of 4 N NaOH. Extract with 2 ml of $CHCl_3$ shaking vigorously. Centrifuge or allow to stand until emulsion is broken. Withdraw 0.5 ml of $CHCl_3$ extract, place in small test tube and add 0.5 ml of ninhydrin reagent. Allow to stand at room temperature for 4 minutes and read. A positive test is evidenced by the development of a deep purple color.

Ninhydrin reagent—0.1 per cent ninhydrin (triketohydrindene hydrate) in $CHCl_3$

Christensen's Citrate-Sulfide Agar

Sodium citrate	3.0	gms.
Glucose	0.2	gm.
Yeast extract	0.5	gm.
Cysteine monohydrochloride	0.1	gm.
Ferric ammonium citrate	0.4	gm.
Monopotassium phosphate	1.0	gm.
Sodium chloride	5.0	gms.
Sodium thiosulfate	0.08	gm.
Phenol red	0.012	gm.
Agar	15.0	gms.
Distilled water	1000	ml.

The medium is sterilized by autoclaving and slanted in the same manner as TSI agar. It should be inoculated by stabbing and streaking. Incubate at 37 C for 5 to 7 days or until a positive result is obtained. Citrate utilization is evidenced by alkalinization of the medium. The H_2S indicator (ferric ammonium citrate and sodium thiosulfate) may be omitted if desired.

KCN Test

Medium

Peptone, Orthana special	10.0	gms.*
Sodium chloride	5.0	gms.
Monobasic potassium phosphate	0.225	gm.
Dibasic sodium phosphate	5.64	gms.
Distilled water	1000	ml.
pH adjusted to 7.6		

*3.0 gms. of Bacto proteose peptone No. 3 may be substituted for Orthana peptone.

The basic medium is sterilized by autoclaving at 15 lbs. for 15 minutes and chilled in the refrigerator. To the cold basic medium is added 15.0 ml of 0.5 per cent KCN solution (0.5 gm. of KCN in 100 ml of chilled distilled water) per liter. The medium is tubed immediately in 1.0 ml amounts in 13 x 100 ml test tubes and stoppered at once with corks soaked in hot paraffin. When stored at 4 C the medium may be used for 30 days after preparation. It should be inoculated with 1 loopful of a 24-hour broth culture, incubated at 37 C and observed for at least 48 hours. Loose corks and cracked tubes must be avoided as they yield false positive results.

All the media described are available in dehydrated form from commercial sources.

Two U. S. Public Health Service scientists who improved methods of identifying bacterial causes of typhoid, infantile diarrhea, food poisoning and other enteric disorders are shown receiving the 1956 Kimble Methodology Research Award for outstanding contribution to the field of public health. Recipients, at the left, are Drs. William H. Ewing and Philip R. Edwards of the Public Health Service, Atlanta, Ga., office. Presenting the award are Dr. Elmer Shaffer, chairman of the awards committee, holding plaque; and Dr. Howard Bodily, chairman of the Annual Conference of State and Provincial Public Health Laboratory Directors. The award, sponsored by the Kimble Glass Company, includes $1,000 and an inscribed plaque. Presentation was made November 12 in Atlantic City, N. J.

EXPERIENCE WITH THE LABORATORY DIAGNOSIS OF ENTERIC DISEASES IN ALASKA*

HAROLD J. FOURNELLE, Ph.D.

Arctic Health Research Center, Public Health Service
Department of Health, Education and Welfare, Anchorage, Alaska

There is general information that enteric infections are common and of frequent recurrence among the Alaskan natives. It has been stated that diarrheal outbreaks occur regularly at certain times of the year, such as during and immediately after spring breakup and during summer and fall camps. It has been said that the great majority of the people, and particularly the children, are affected at those times. Williams[1] and Pauls[2] have published accounts of what is known about *Salmonella* and *Shigella* infections in Alaska. According to the available records, *Salmonella typhosa* had been reported most frequently with salmonellae accounting for 60 to 70 per cent of the reported isolations. Of the *Shigella*, Flexner and Sonne types were reported most often.

Although this gives some indication of what occurred in the past, it does not tell us what the prime causative agents of diarrheal infections are today. With respect to enteric parasitic infections, still less is known. The only published reports known are those of Dorothy Hitchcock[3,4] who made a study of Eskimos in Bethel and Kotzebue in 1949 and 1950, respectively. She stated that the incidence of intestinal protozoa was similar to that of the general population in the northern part of the United States. Few *Endamoeba histolytica* were found. *Diphyllobothrium* sp., or fish tapeworm, was observed in 15 and six per cent of the people, respectively, in the two villages studied.

The United States Armed Forces Epidemiological Board considers intestinal pathogens a problem of importance in arctic regions, and has sponsored an extended study of the epidemiology of these infections in those areas. The study was initiated in Alaska in 1954. Primary interest was in the mode of transmission of these diseases, i.e., whether it was vehicular or by contact. Although the study[5] was limited with respect to time, it did indicate that the problem at the time appeared to be one of shigellosis and that transmission appeared to be through a person-to-person contact.

The survey which the Arctic Health Research Center is carrying on is concerned with both the bacteriological and parasitological aspects of enteric infections in an Eskimo population of the lower Kuskokwim River area in southwestern Alaska. The purpose of the survey is to determine (1) the prevalence of reported diarrhea and actual infections, (2) the age groups involved, (3) the time of year of occurrence, and (4) the bacterial and parasitic agents involved. The survey will be extended to other Alaskan

* Portion of paper presented at the symposium held at the First North American Conference of Medical Laboratory Technologists at Quebec City, Quebec, Canada, on June 18, 1956.

areas and will also include other native groups. The Alaskan natives are comprised of three groups, viz., Eskimos, Indians, and Aleuts. They number approximately 18,000, 15,000, and 2,000 respectively. Our work in the field has been tied in to some extent with that of the Native Village Sanitation studies whereby a concerted effort is being made to improve sanitary conditions in the Eskimo villages in the lower Kuskokwim and Yukon River areas. It is hoped that an improvement in household and general village sanitation will, in time, be reflected in a drop in the rates of communicable diseases. We are also endeavoring to see if some bacterial or parasitic organism or organisms can be used as a yardstick to measure the effect of environmental sanitation on the health and general well-being of the people.

From the existing reports of the high prevalence of diarrheal diseases among these people, we thought that possibly there would be an appreciable carrier rate.

To date the survey has covered seven villages with a total population of a little over 2,000. Village size varied between about 150 and 600 people. The general area covered is around the village of Bethel which is some 400 air miles west of Anchorage. Village locations were coastal, tundra, and river.

It was decided at the start that it would probably be most advantageous to take the laboratory into the field where the specimens could be cultured and where initial isolations of bacteria could be made. There were several reasons for this, viz., (1) to eliminate the prolonged delays which often occur in Alaska where material is shipped to the laboratory for examination, and (2) to eliminate the use of preservatives which are not satisfactory for many of the *Shigella*. It was considered that the culturing of fresh stools would facilitate the isolation of enteric bacterial pathogens, especially certain of the shigellae, and particularly where pathogens were few in number.

The matter of whether or not rectal swab specimens would be preferable to passed whole specimens has been considered. There is no doubt that for sampling certain groups, such as military personnel and children, and for large numbers, it is a fast and efficient method, and, further, it permits the taking of specimens at will without having to wait for the passed material. However, if a fresh stool specimen can be cultured I believe it to be as good as a swab specimen. It was believed that it would be easier to get a stool specimen, especially from the older people, than it would be to take a swab specimen. Another reason for using the whole stool is that two to three grams of the specimen are needed for the parasitological examination. This portion is placed in M.I.F. (merthiolate-iodine-formaldehyde) preservative-stain fluid[6,7] and held until return to the main laboratory.

The fact that dogs play an important part in the life of the

Eskimos, and the fact that there are usually as many or more dogs in a village as there are people, and, further, since dogs are known to be carriers of salmonellae, they were considered as possible sources of human infections, and were therefore included in the survey.

The field laboratory is entirely portable. It is necessary that the various units of equipment and supplies be kept down in size and weight, particularly since the material must go into a small bush plane once we are in the field. The equipment consists of two small electric incubators, Petri plates and bottled media for culturing 400 to 600 specimens per trip, T.S.I. agar slants in screw-capped test tubes, M.I.F. fluid in small, screw-capped vials, specimen cartons, and the other items required for bacteriological work. Selenite broth has been used for enrichment in some instances. In order to sample a larger number of the people on each visit, enrichment is seldom used, and only direct culturing is usually done. Sterile cotton-tipped swabs are used to streak agar plates. Specimens are collected in one-half pint cardboard water-proof containers. The inoculating wire (for picking colonies) is sterilized with a propane gas torch which has been found to be more satisfactory than the alcohol blast burner. For the parasitological specimens, 10-dram vials containing 9.4 ml. of a merthiolate-formaldehyde mixture are used. Just before adding the specimen, 0.6 ml. of Lugol's iodine solution is added to the vial from a burette. The specimen is broken up as completely as possible. The entire equipment and all supplies are usually contained in 17 or 18 packages, the whole setup weighing somewhere between 1,000 and 1,100 pounds.

Before the field trip can be made, it is necessary to contact the Area Office of the Alaska Native Service in Bethel to make arrangements for going into the various villages. The laboratory is usually set up in the Alaska Native Service school which is about the only place in each village with suitable accommodations, both for the laboratory work and for boarding the field worker. The schools are equipped with small electrical generators. A certain amount of improvisation with respect to maintaining a satisfactory incubation temperature for cultures is necessary because the generators are turned off at night. Inoculated agar plates and slants are placed overnight in a room heated with an oil burning stove. Satisfactory temperatures have thus been maintained. The school teacher usually is a reliable source of information on general health conditions in the village.

Upon arriving at a village and after calling on the teacher, my first step is to contact the Village Council which is made up of a chief and several male members who are all elected by the villagers. I tell these people what I want to do and I ask their

permission to carry it out. It has always been given.

The next thing is to hire an interpreter. Usually it is a young person, male or female, who understands and speaks English as well as his own language. Visits are made to the homes where, by means of interviews usually with the housewife, information regarding diarrheal illnesses is obtained. The information requested includes: names and ages of every one living in the household, when each had diarrhea last and how they were affected. Time of recollection is limited, usually going back to the last spring breakup. Specimen cartons are marked with the names of the individuals living in the house and instructions are given that the specimens are to be taken to the field laboratory soon after they are passed. Two or three containers are also left for dogs with the request that only fresh material be taken. As a rule, I have found the Eskimo people to be very cooperative.

Other information noted at the time of interview are the number of rooms in the house, in order to arrive at an index of crowding, and the degree of cleanliness. With respect to sanitary facilities as we know them, they are, in general, almost entirely lacking. Sources of water are nearby streams, sloughs, or ponds. A limited number of water samples are taken from these sources, also from water barrels or buckets in the homes, and coliform tests are made.

Within the household, human wastes are collected in a variety of receptacles ranging in size from a one pound coffee can to a five gallon gasoline can with the top cut off. These containers, along with kitchen wastes, are most often dumped indiscriminately near the house. Efforts are being made by the sanitary engineers and sanitarians to correct this practice through the use of subsurface disposal pits located away from the living quarters. Very often the dogs are tied up near the houses, and this helps to contribute to the accumulation of excrement about the houses. The people are now being asked to keep the dogs some distance from the village.

Culturing of stool specimens has been limited, for the most part, to streaking onto SS agar. As mentioned earlier this was done in order to permit the sampling of as many of the population as possible. In the case of infant diarrhea, culturing was also done on a less inhibitory medium such as MacConkey's agar for pathogenic *Escherichia coli* types. Suspicious colonies appearing on SS agar are picked to T.S.I. agar slants and those giving reactions expected of *Salmonella* and *Shigella* are saved while others are discarded. This has been the extent of the bacteriological field work.

Definitive biochemical and serological studies are made upon return to the laboratory in Anchorage. Ordinarily cultures isolated in the field and which are believed to resemble *Salmonella* or *Shigella*

are transferred to nutrient agar slants or deeps and held at a low temperature, around 50° F. or lower. The first examination consists of streaking urea agar slants in the search for *Proteus* organisms and discarding all positives. Usually the biochemical tests are performed before any serological typing is done since we are not especially concerned with rapid reporting of results. An initial set of biochemical reactions is first determined, and later, along with the agglutinating studies, a few additional tests are done. Initial biochemical reactions consist of fermentation of glucose, lactose, sucrose, and salicin; motility, indol production, and methyl red and Voges-Proskauer reactions. Where hydrogen sulfide production appears weak, delayed, or in doubt on the T.S.I. agar slant, a recheck is made on Difco's peptone iron agar. I have found this medium very satisfactory for the determination of hydrogen sulfide. Additional media for *Shigella* differentiation are rhamnose, xylose, and dulcitol broths. Those cultures which give biochemical reactions characteristic of *Salmonella* and *Shigella* are run through the respective polyvalent, group, and type antisera. A certain number of confirmations are requested of the Public Health Service's Communicable Disease Center at Chamblee, Georgia, as well as assistance in identification of organisms we cannot type and in the determination of phage types of *S. typhosa.*

Interview data obtained on some 1370 individuals to date, show that approximately 38 per cent have a history of diarrhea, the majority of cases having been reported within four months prior to the time of the interview. The range of reported prevalence for the seven villages varied between 19.0 and 57.3 per cent. As time increased beyond four months of the interview, low morbidity rates, some of which were less than 1.0 per cent, were reported. The data also showed that approximately two-thirds of the histories of diarrhea were reported in the under-11 years age group.

Bacteriological examination of fecal specimens from approximately 1030 individuals showed that the predominant pathogens were two types of *Shigella flexneri,* viz., 2a and 3. A few *Sh. sonnei* and *Sh. alkalescens* were also found. Although there are believed to be many typhoid carriers among these people, only a few were detected. Some difficulty has been experienced with getting phage types on these organisms. Cultures are held for some time before they can be identified and sent to the Communicable Disease Center in the states for phage typing. As a result, reports have shown degraded Vi forms. Village enteric infection rates ranged as high as 11.2 per cent and showed an over-all rate of 4.5 per cent. Approximately two-thirds of the actual infections were found in the under-11 years age group. About one-third of the isolated organisms were from individuals who reported no diarrhea. For the most part, isolations of

pathogens were from individuals who were without symptoms at the time of interview. In regard to recency of diarrhea in persons from whom bacterial pathogens were isolated, all who reported diarrhea did so within the four-month period prior to interview, and slightly over two-thirds reported diarrhea within the month prior to questioning. Two *Escherichia coli* types were taken from three young Eskimo children who were patients at the Bethel Native Hospital. The types were 055:B5 and 0117:H27.

According to interview data on morbidity reporting and on recency of reported diarrhea in those from whom pathogens were isolated, diarrhea was most prevalent during the months of July and August.

Of the 172 dog fecal specimens cultured, only one bacterial pathogen was found. It was *Shigella flexneri* 3 and was found in the village with the highest human infection rate. No salmonellae were isolated. In contrast to our findings, workers[8] from the Arctic Aeromedical Laboratory at Ladd Air Force Base, near Fairbanks, Alaska, found *Salmonella typhimurium* in both dogs (7 per cent) and humans (12 per cent) at Barrow in 1954 and 1955. They stated that, in the case of the dogs, these organisms were isolated mainly from frozen fecal specimens. At Fort Yukon,[9] in 1955, *Salmonella typhimurium* was found in 6 of 100 dogs examined.

Parasitological examinations were made of 1035 human and 174 dog fecal specimens. Both a direct and concentrated procedure[10] were employed. In humans, significant protozoan and helminth infections were indicated by the number of *Endamoeba coli* and *Diphyllobothrium* sp. found. *Endamoeba coli* ranged from 14.3 to 41.3 per cent in the seven villages, and showed an over-all average of 23.3 per cent. A total of seven individuals were found infected with *E. histolytica*, giving a rate of 0.7 per cent. The very low prevalence, i.e., 0.6 per cent, of pinworm (*Enterobius vermicularis*) was no doubt due to the method of collection of the specimens. Other protozoan infections were low. Two types of *Diphyllobothrium* sp. were found. One of these showed ova which averaged about three-fourths again the size of the other, both in length and breadth. The species are not known since only the ova were observed. The dimensions of the smaller of the two approximated those of *D. latum*. Human prevalence per village ranged from 1.7 to 33.6 per cent, with an average of 14.2 per cent. Three individuals exhibited both the regular and large size ova. The large size ova were found in two villages only and made up 27.3 and 36.7 per cent of the total *Diphyllobothrium* ova seen in those two villages.

In dogs, the only protozoan detected was *Endamoeba coli* which amounted to an over-all average of 4.6 per cent. Significant numbers of helminths were hookworm and fish tapeworm (*Diphyllobothrium* sp.). The prevalence of hookworm ranged from 15.3 to 66.7 per cent, with an average of 53.4 per cent. *Diphyllobothrium* sp. (both

large and regular size ova) ranged from 0.0 to 43.7 per cent, with an average of 29.3 per cent. The large size ova were found in dogs in three villages. The only other parasite found was *Ascaris* sp., showing a range of 4.3 to 14.3 per cent and averaging 6.9 per cent.

Something should be said about the M.I.F. technic. For our purpose, i.e., shipping fecal specimens from the field to the laboratory for parasitological examination, we have found it to be very satisfactory. We have held some specimens in the M.I.F. fluid for close to five months before examining and have recovered a variety of protozoan cysts and helminth ova in excellent state of preservation. In addition to being preserved, cysts and ova are stained and show no distortion of morphology. Internal structures are well differentiated. Amoebic trophozoites show up very nicely. We believe that both direct and concentration procedures are necessary. Since it is practically impossible to examine stool specimens as soon as they are brought to the laboratory, the use of a satisfactory preservative-stain which will permit delayed examinations will appeal to many.

In addition to the enteric bacteria and parasites, we have also kept in mind another bacterial type, the enterotoxin-producing micrococcus, which is frequently the cause of diarrhea, as well as of other symptoms, and which accounts for many food poisoning outbreaks. A medium for detecting this organism is also taken into the field. However, I have not had occasion to make examinations for this organism.

In summary, the survey has shown that the diarrheal problem among the Eskimos in the villages visited has been one of shigellosis, with two types of *Shigella flexneri*, viz., 2a and 3, predominating. The age group most seriously affected were the children 10 years of age and under, who accounted for approximately two-thirds of the reported cases of diarrhea and of the isolated bacterial pathogens. Except for a few *Salmonella typhosa*, no other salmonellae were isolated. According to the morbidity reports, about 38 per cent of the people gave a history of diarrhea, with the greatest prevalence occurring during July and August. So far dogs have not been found to be important as carriers of bacterial pathogens.

In regard to parasitic infections in humans, *Endamoeba coli* and *Diphyllobothrium* sp. were most numerous. Two types of *Diphyllobothrium* were found in two of the villages. The prevalence of *E. histolytica* was very low, averaging 0.7 per cent. In dogs, the most numerous parasites were two helminths, viz., *Diphyllobothrium* and hookworm.

It will be some time before a complete understanding of the causes of diarrhea in Alaskan natives will be gained. It is going to take considerable effort to bring that about, but we feel that we have made a beginning.

REFERENCES

1. Williams, Ralph B. Summary of Salmonella and Shigella of Alaska. Northwest Medicine, **49**(5): 340-341, May, 1950.
2. Pauls, Frank P. Enteric Diseases in Alaska. Arctic, **6**(3): 205-212, Oct., 1953.
3. Hitchcock, Dorothy J. Parasitological Study on the Eskimos in the Bethel Area of Alaska. Jour. Parasit., **36**(3): 232-234, June, 1950.
4. ————————. Parasitological Study on the Eskimos in the Kotzebue Area of Alaska. Jour. Parasit., **37**(3): 309-311, June, 1951.
5. Gordon, John E., Frank L. Babbott, Jr., and Joan Griggs Babbott. Transmission of Intestinal Pathogens in Polar Climates. Arctic Intestinal Disease Studies. Ann. Rept. to Commission on Environmental Hyg. of the Armed Forces Epidemiological Board. For period, Mar. 1, 1955, to Feb. 28, 1956. 44 pp., 1956.
6. Sapero, James J., Deaner K. Lawless and Cloyce P. A. Strome. An Improved Iodine-Staining Technique for Routine Laboratory Diagnosis of Intestinal Protozoa. Science **114**(2969): 550-551, Nov. 23, 1951.
7. Sapero, James J., and Deaner K. Lawless. The "MIF" Stain-Preservation Technic for the Identification of Intestinal Protozoa. Amer. Jour. Trop. Med. & Hyg., **2**(4): 613-619, July, 1953.
8. Grumbles, L. C., and John A. Maciolek. The Isolation of Enteric Pathogens from Humans and Dogs at Barrow, Alaska. Paper presented at 6th Ann. Alaska Science Conference, Fairbanks, Alaska, June, 1955.
9. Cullison, Jack W. Personal communication, 1956.
10. Blagg, W., E. L. Schloegel, N. S. Mansour, and G. I. Khalaf. A New Concentration Technic for the Demonstration of Protozoa and Helminth Eggs in Feces. Amer. Jour. Trop. Med. & Hyg. **4**(1): 23-28, Jan., 1955.

The letter from Dr. Fournelle, which accompanied his paper, adds an interesting note. (He borrowed a typewriter from the anthropologist in the village of Napaskiak, Alaska, where he was working from July until "early September" and where he "will return each month, until June 1957, for 8 to 10 days.")

Dr. Fournelle said:

"Of course I'm running into problems in setting up the house for living quarters and lab work. We are renting the house from an Eskimo. As Eskimo dwellings go, it is a pretty good place. However, it's not the Chateau Frontenac. The water supply is rain water which is caught in oil drums (which have to be thoroughly cleaned first and which is quite an operation). When that runs out, then it's the Kuskokwim River. Boiling or chlorination is necessary. I have a chemical toilet, which is quite a luxury here. A gasoline camp stove is used for cooking and a Coleman lantern for light. Also, have a small electric light plant (750 watts) to furnish electricity for the incubators and when in operation will provide light for the house. It is not run continually, however. The house will be heated with oil. Gasoline and motor oil for the light plant and stove oil for heating are obtained at Bethel which is about 5 miles upstream. It is brought in by boat. Most Eskimos have either 25 or 30 H. P. outboard motors. They don't go in for the smaller motors."

Anyone interested in going to Alaska?

EXPERIENCE WITH THE LABORATORY DIAGNOSIS OF ENTERIC DISEASE IN CANADA

W. R. BAILEY, Ph.D.**
Bacteriologist in Charge, Enteric Bacteriology Laboratory, Laboratory of Hygiene, Department of National Health and Welfare, Ottawa, Ont.

The National Salmonella and Shigella Typing and Reference Centre for Canada receives for the most part cultures which have been isolated from cases of enteric disease throughout the country, as well as isolates from animals and poultry infections. The majority of these strains have been provisionally identified by the submitting laboratories with antisera supplied by the National Centre.

The condition in which cultures are received varies from good to bad. A loose fitting cotton plug without protective covering is a poor and dangerous method of closing a culture for shipment. Proper shipping containers are required by the postal authorities. Broken tubes or contaminated plugs constitute a definite hazard to all handlers from the date of shipment to date of receipt. Paraffined corks or screw caps are recommended for closing culture tubes, while firm agar, preferably in stab form, or dorset egg in slope form are desirable as transport media.

Blood and fecal specimens are occasionally received. Such specimens should also receive proper attention when being packaged for shipment. Fecal specimens from local sources may be submitted in waxed cardboard containers if they are delivered by messenger within two hours of collection, or suspended in glycerol buffer in properly stoppered bottles if longer distances or longer time lapses are involved.

Another method of stool collection that has been found to be quite suitable in Salmonella infections is the filter paper method (Can. Jour. Pub. Health, Dec. 1953) especially when long distances and poor facilities exist between shipping and receiving points.

Cultures take from one to four days to reach the laboratory in Ottawa, varying with the shipping distance, and the identification report ordinarily goes out within forty-eight hours. When rapid identification is required, cultures are shipped via air mail and the report goes out by telegraph.

To aid the various Provincial and D.V.A. hospital laboratories in provisionally identifying Salmonella, Shigella and *E. coli* isolates, the National Centre supplies certain polyvalent and monovalent antisera that will enable tentative identification of the more common types encountered. These sera include those for the identification of *E. coli* types associated with infantile diarrhea.

* Portion of paper presented at the symposium held at the First North American Conference of Medical Laboratory Technologists at Quebec City, P. Q. Canada, on June 18, 1956.
** Now at the University of Delaware, Wilmington, Del.

Salmonella

To date sixty-seven different Salmonella types have been reported in Canada. There are presently about five hundred serotypes officially recognized. Annually, about thirty different serotypes are represented, and each year for the past six years some type not previously reported in the country has been identified. It is seldom that epidemiological investigations reveal the source of such strains and it is of interest to note that some of these make only one appearance while others may persist and become common types.

Of the sixty-seven types reported, the ten most commonly found during the last six years in 3,795 human infections and 995 animal infections are shown in **Table 1,** which gives the incidence of these types.

Table 1. Most common Salmonella types in Canada 1950-1955

Human Strains		Animal and Poultry Strains	
1. S. typhi	43.0%	S. typhi-murium	31.6%
2. S. typhi-murium	23.6%	S. thompson	14.0%
3. S. paratyphi B	12.3%	S. bareilly	12.6%
4. S. newport	4.4%	S. gallinarum-pullorum	11.9%
5. S. heidelberg	3.1%	S. oranienburg	6.4%
6. S. oranienburg	2.2%	S. bredeney	4.9%
7. S. tennessee	1.9%	S. heidelberg	2.4%
8. S. bareilly	1.6%	S. kentucky	2.2%
9. S. thompson	1.4%	S. derby	2.1%
10. S. montevideo	1.0%	S. newport	1.5%

Number of strains investigated Human—3,795 Animal— 995

S. typhi—Although reported in all provinces, over 60% of the reported cases occur in the province of Quebec. The rural areas show the highest incidence. More than twenty phage types have been encountered, of which phage type E_1 is the most prevalent. Types C, A, D_1 and F_1 follow in that order of incidence. Two new phage types have been isolated in the last two years, both of which are awaiting official recognition by the International Centre.

S. typhi-murium is widespread in all provinces. It is the most common in animal infections and the second most common in human infections. It is the most common Salmonella encountered in gastro-enteritis.

S. paratyphi B infections usually show a clinical syndrome of paratyphoid fever but the type is also found in infections of the g.e. type. Although the figure may not be accurate, it would appear that approximately 12% of the strains isolated from g.e. infections are tartrate positive. *S. paratyphi B* typical strains are tartrate negative, produce a slime wall and cause paratyphoid fever. An appreciable number of the strains received are monophasic on arrival at the laboratory. During 1955, three of the ten recognized phage types were encountered. These were types 3a, 3b and

Taunton, with type 3a being the most common.

S. newport has its highest incidence in the province of Quebec, although it is reported in all provinces with the exception of Prince Edward Island.

S. heidelberg was isolated in Canada for the first time in 1952. Its first appearance was in chickens then in humans in Alberta. It has spread to all provinces with the exception of New Brunswick and P.E.I. to date, and is now the fifth most common type encountered in human infections and the seventh most common in poultry infections.

S. oranienburg has been found in all provinces but P.E.I.

S. tennessee has been most commonly found in Ontario and so far has not been reported in Manitoba, New Brunswick or Newfoundland. It is not a common pathogen of the lower animals.

Alberta shows a fairly high incidence of *S. bareilly* in poultry infections.

S. thompson is responsible for about 14% of the Salmonella infections in poultry in Canada with Alberta showing the highest incidence. Strangely enough it is still quite rare in the U. S. A. Great Britain also shows a high incidence in poultry.

S. montevideo is the tenth most common Salmonella in human infections but a rare animal pathogen in Canada.

S. gallinarum-pullorum is almost strictly a pathogen of poultry but there have been a few cases of human infection and one reported isolation from a dog.

S. bredeney is fairly common in poultry, swine and sheep infections but not a common human pathogen in Canada even though it has been the cause of a few minor outbreaks of diarrhea in small communities in the larger provinces.

S. kentucky and *S. derby* are found more commonly in poultry than in human infections.

Shigella

Since 1952 our records show that nine types have been encountered in Canada. **Table 2** shows the absence of any Group A shigellae. The representatives of Groups B, C and D are listed showing the incidence of the more common types.

Table 2. Shigellae reported in Canada 1952-1955

Type			Incidence
B. Sh. flexneri	1b	I: 4,6	
	2a	II: 4	..... 4.4%
	3	III: 6, 7, 8, 9	..... 5.4%
	4b	IV: 6	
	5	V	
	6	VI	..... 0.9%
	X	—: 7, 8, 9,	
	Y	—: 4	
C. Sh. boydii	2		
	9		
D. Sh. sonnei			.....88.7%

Sh. sonnei has been responsible for 88.7% of the Shigella infections during the last four years. It has shown an increase during this period and has to a large extent replaced the Flexner types as the principal causative agent of Shigellosis in this country. Such a trend has also been reported by Bradley and other investigators in Great Britain. Records of infections have indicated that *Sh. sonnei* is spread more by personal contact in institutions than by such vectors as water and flies. This Shigella type has been found in all provinces of Canada with the highest incidence occurring in British Columbia and Alberta. Mixed infections with other enteric pathogens such as *S. paratyphi B, S. tennessee* and *Alkalescens-Dispar* O_1 *have* been reported in cases of gastro-enteritis.

Sh. flexneri types 2a, 3 and 6 are the most commonly reported members of Group B, while type 4b has been reported only in monkeys being used for polio vaccine work. *Sh. flexneri* "X" and "Y", considered as loss variants, are probably degraded forms of *Sh. flexneri* 3 and 2a respectively.

Enteric Infection in Canade Due to Salmonella and Shigella

For the period 1951-1955, human infections with Salmonellae showed a marked increase during the month of June reaching a peak in August. The incidence of infection, however, remains relatively high in September and October but falls off during the winter months to approximately 50% of the level experienced during the summer months. Animal and poultry infections show a marked increase during May, reach a peak in July and then fall off during August and September until a relatively low level of infection is observed during the winter months.

By comparison, Shigella infections in Canada during approximately the same period, do not attain a peak during the warm months. They are encountered the year round, but perhaps show a higher incidence during the winter months as evidenced by the number of institutional outbreaks, due mostly to *Sh. sonnei.*

Enteropathogenic E. coli

It is only within the last two years that the laboratory in Ottawa has been receiving cultures and reports of isolations of *E. coli* strains associated with outbreaks of diarrhea. **Table 3** lists the number of isolations of types encountered during 1955. The incidence is that found in children.

Nova Scotia showed the highest incidence of *E. coli* 055:B5 which headed the list of *E. coli* types causing child diarrhea. The pathogen was responsible for outbreaks in babies' wards in various hospitals. Ontario and Nova Scotia show the widest distribution of types in the country, while Ontario was the only province in which all of the types listed were found during the year. Type 0127:B8 was isolated for the first time in 1953 in British Columbia and simultaneously in two well separated states in the U. S. A. Recently a

single isolation of type 0128 was reported in Manitoba, bringing us to a total of eight types among those reported by Ewing and others to be associated with this childhood disease.

Table 3. Enteropathogenic E. coli types in Canada during 1955

Type	No. of Cases	Incidence
E. coli 055:B 5	68	47%
026:B 6	29	20%
0111:B 4	23	16%
0127:B 8	8	5.5%
0126:B16	8	5.5%
0125:B15	5	3.4%
086:B 7	3	2.0%

Alkalescens-Dispar

Only one member of this group has been encountered in Canada, according to our records. This is *Alkalescens-Dispar* O_1, which has been isolated mostly from babies suffering from diarrhea. There have been a few instances of isolations being made from the nose and throat of babies.

Summary of Observations and Recommendations

There are certain problems that confront laboratory personnel working in enteric bacteriology and some of these problems are created in the initial approach to the diagnosis of the disease during the isolation of the inciting agent. The first step and one of the most important is the collection of the specimen and this unfortunately does not always receive the attention to detail it deserves. As laboratory people we can only report what is found in the specimen submitted. The failure to isolate the causative agent in an infection may be due to an improperly taken specimen or perhaps due to a lack of information on the clinical condition of the patient for guidance in outlining laboratory procedure.

Specimens should consist of an adequate amount of material and naturally should reach the laboratory as soon as possible. They should be taken before treatment with sulphonamides or antibiotics but if this is not always possible, the specimen should be so labelled as to indicate its possible contents in order that suitable precautions may be taken by the laboratory. An example of this could be the treatment of a blood specimen taken from a patient that had received sulpha drugs or antibiotics: the addition of para-aminobenzoic acid to the medium will counteract the bacteriostatic action of sulphonamides, while the addition of cysteine will inhibit the action of streptomycin. Other antibiotics cannot be counteracted in this way and dilution procedures are used when practical.

The stage of the disease is of equal importance and to this end clinicians are expected to take into account the type of

specimen that is most likely to yield positive results during a particular phase of the disease, and further realize that when this selection is not made, negative findings do not necessarily indicate an incorrect clinical diagnosis. A good example of this is afforded by stool, blood and urine specimens in typhoid fever. The submission of samples of blood serum for the Widal test when antibody response cannot be expected is perhaps another mistake.

In so far as desirable media for plating specimens is concerned, various laboratories have their preferences and today we have a wide choice. It is, however, safe to recommend the use of two media:—one highly selective, such as S.S. agar or Bismuth sulphite agar or Desoxycholate-citrate agar, and another less inhibitory medium such as MacConkey agar or EMB agar. Blood agar is also used in the diagnosis of *E. coli* infections. The use of enrichment media such as Tetrathionate and Selenite broths is recommended at the time of the direct plating procedures when fecal material from cases of Salmonella and Shigella infection is being examined.

Problems arise in the selection of colonies for examination and poor technique breeds problems. The necessity for discrete colonies attained by proper plating technique is most important during this phase of the procedure. Colonies are best selected with the aid of a dissecting microscope using a magnification of 10X and an oblique lighting system. Under no condition, when picking colonies, should one test the temperature of a flamed needle by touching it to a seemingly colony-free area on the plate before making the selection. This often leads to the contamination of a culture with organisms that are only inhibited by the bacteriostatic action of the medium.

In most laboratories today suspicious colonies are transferred to slopes of Triple Sugar Iron agar for screening. Suspected Salmonellae, Shigellae and others may be further screened by seeding to urea agar, KCN medium and phenylalamine agar. The last mentioned medium will screen out those organisms that convert phenylalamine to phenyl pyruvic acid—namely Proteus and Providence (this is the A.P.P. test of Buttiaux). Proteus splits urea, Providence does not. The KCN medium is extremely useful in further differentiating between Ballerup Bethesda and Arizona and other groups. We have confirmed, to our complete satisfaction, the observations of Braun, Moller and Edwards on the KCN test and those of Buttiaux and others on the APP test and therefore recommend these two tests for routine use.

Mixed cultures of spreading Proteus and Salmonella or Shigella are often received and may be separated in the laboratory by plating the mixture on 5% agar. This concentration of agar pre-

vents the swarming of proteus and allows the pure selection of the desired organism by examining discrete colonies.

Mixed cultures of Salmonellae have been received for identification and it is our experience that if such a mixture be cultured in plates of semi-solid agar containing antiserum for one of the components, the other component will swarm through the agar and be recovered in pure form.

Non-motile strains of Salmonella, other than *S. gallinarum-pullorum* have been isolated from cases of infection here in Canada. Such strains, especially if lacking flagella or possessing only a few non-functional flagella cannot be serotyped due to the inability to recognize the flagellar antigens. By transduction with the appropriate bacteriophage, such strains can be rendered motile and thus typable. The antigens recognized are usually those that are in a latent state in the non-motile form.

Other problems encountered are antigenic cross relationships in the serodiagnosis of types. The development of both phases in some diaphasic Salmonellae strains often presents difficulties and those with which we encounter difficulty are *S. paratyphi B*, *S. bredeney*, *S. javiana* and *S. cholerae-suis*.

Apart from certain pathogenic enterobacteriaceae that are found almost exclusively in warmer climates and as examples I may cite *S. paratyphi A*, *Sh. dysenteriae* 1 (Shiga) and other Group A Shigellae, we find a similar pattern of the common Salmonella and Shigella types that are encountered in other countries. The high incidence of *Sh. sonnei* infections in Canada in the past few years has been noticeable but there again similar trends have been reported in milder climates. Generally speaking, therefore, the cold climate aspect does not materially alter the pathogenic picture for the Enterobacteriaceae.

Before closing, I would first like to comment on one phase of the laboratory diagnosis of enteric infections which I have so far neglected to mention. I refer to the determination of agglutinins in the patients serum by the Widal test. This test does not perhaps occupy the position of importance that it once held but there are many laboratories that still use the test as an index of typhoid or paratyphoid infection. Here in Canada all of our provincial laboratories perform routine Widals. For this reason some years ago, at the request of the directors of these laboratories and D.V.A. hospital laboratories, our department started preparing standard antigens. Today we distribute nine antigen suspensions which are standardized for density and agglutinability, thus providing each laboratory with the same material and therefore making it possible for them to obtain comparable results. We share the late Dr. Felix's views on the need for standard antigens and a standard procedure. The antigens supplied by our division are checked by a standard test before distribution and periodically throughout the year. Only by adopting

standard procedures do we feel that different laboratories can make an intelligent interpretation of the test.

In addressing you, the medical technologists, who are doing these tests in your laboratories, may I point out that there is more to the Widal test than the mere expedient of adding antigen to a series of serum dilutions. In the standardization of an antigen suspension, especially of *S. typhi*, there are certain factors that should be taken into consideration. One of these is the pH level used. Too low or too high a pH will depress agglutinability. Levels above pH 7.0 markedly lower percentage agglutination. Other factors of density, salt concentration etc., will also affect agglutinability titres.

In closing then, may I say that we at the National Centre enjoy a happy and cooperative relationship with the Provincial Department of Health. Insofar as is possible these laboratories provide case histories with the cultures submitted for identification and the pooled information may be used in epidemiological investigations. This cooperation enables us to put out an annual report that summarizes the enteric bacteriology picture showing the incidence of these infections in Canada.

SYMPOSIUM DISCUSSION

By DR. E. T. BYNOE, Ph.D.

Chief Bacteriological Laboratories, Laboratory of Hygiene, Dept. of National Health and Welfare, Ottawa, Ontario, Canada

Mr. Chairman, ladies and gentlemen.—First, I would like thank the Committee who invited me to summarize the symposium. Dr. Bundock has already extended the best wishes of the Department of National Health and Welfare to your first North American Conference. I would like to add the good wishes of the Laboratory of Hygiene for the success of this Conference. You were extremely fortunate in having had such internationally famous authorities in the Salmonella-Shigella-Escherichia field as Dr. Kauffmann and Dr. Edwards address you. We owe sincere thanks to Lederle Laboratories who made this possible. As I listened to the very interesting addresses of the speakers, I jotted down a few items which I thought to be some of the highlights of this symposium.

First of all, in reference to enteric disease in cold climates, there does not seem to be much difference in the etiology of enteric disease as it occurs in cold climates (in Northern Canada and Alaska) and in the southern parts of the Continent. Cold certainly does not seem to kill bacterial pathogens and, of course, this has been known for many years. In a classical outbreak of typhoid fever in the Spring of 1885 in Plymouth (Pa.), out of a total population of 8,000 people, 1,104 contracted typhoid fever and 114 died. This outbreak was, clearly and convincingly, traced to a man who developed typhoid fever early in the winter and whose dejecta were thrown out on the snow and frozen ground. With the melting of the snow in the spring, this was washed into one of the streams feeding into one of the main reservoirs, thus contaminating the main public water supply. In this instance, the typhoid organisms remained viable in their fecal environment at freezing temperatures for over three months. We cannot rely on cold and low temperatures to kill pathogenic organisms.

Dr. Fournelle's reference to his work in the Arctic is extremely important. The Arctic and northern Canada are becoming more and more important, both strategically, from the point of view of national defence and also, economically. More and more people are moving into our Northland and I think it only fit and proper that we should know something of the health situation and hazards which they are going to have to face. Dr. Fournelle's actual findings are perhaps low in regard to the bacterial pathogens found in these northern regions, as reported by some others. There has been some work done in Canada by Dr. T. W. M. Cameron of the Institute of Parasitology, and his associates, on the parasites of the North lands. A survey by Wolfgang (1954) and examination of the hospital records of five hospitals serving our northern inhabitants, showed 102 or 7.5% of 1,347 patients in hospitals carrying *Diphyllobothrium latum*. *E. histolytica* has been reported much more commonly than the 0.7% rate, however, reported by Dr. Fournelle for Alaska. Dr. Cameron estimates that about 2% of our northern population is infected with this parasite, and Miller, in 1952, in a survey conducted in northern B. C. found that as high as 8% of the children examined were infected with *E. histolytica*. Pinworms were particularly common. Almost all of the Indian and Eskimo children that Miller examined were infected with the parasite and *Giardia lamblia* was also very common. One of our problems in the North is hydatid disease. This is particularly common, and the echinococcus is well established in the Arctic and sub-Arctic fauna—moose, caribou and dogs. In the Arctic, despite the plane transport that the U. S. Public Health Service

might offer, the principal means of transport in the winter is still dogs. Dogs have been shown to carry quite a varying and heavy load of parasites and bacteria. Although Dr. Fournelle found only one bacterial pathogen in 172 dogs examined, there are other figures which show a much higher incidence of Salmonellae in the dog. He referred, I believe, to the work of Grumbles in 1955, in Alaska, who found 7% of the dogs examined, to be carrying *S. typhimurium*. Dogs, moreover, are the principal sources of many parasitic infections. Where contact is so close between man and dog, as exists in the North, naturally any infection of the dog presents a serious threat to man.

A few remarks now with regard to techniques. I suppose, as technologists, you are more concerned with techniques than epidemiology. As Dr. Bailey mentioned, the best laboratory result will be only as good as the condition of the specimen received allows. While the transport of specimens is not a particular problem in the clinical (hospital) laboratory, where the laboratory is usually in close proximity to the patient, in public health laboratories, things are quite different. Here, specimens may sometimes take days to reach the lab. In these instances, it is very important that the transport medium should be one that will preserve, as closely as possible, the bacterial flora in the freshly collected specimen. Dr. Bailey referred to the filter paper technique as a useful one. L. K. Joe and his co-workers (Am. Jour. Trop. Med. Hyg., 1956, **5**: 133) have made similar observations. There is another method that has not been mentioned, which I would like to call to your attention. Dr. Stuart, Director of the Provincial Laboratories, Alberta, recently described a transport medium or method which is worthwhile investigating. The method is based on two premises. One is that the pathogens in the specimen die during transport due to the effects of oxidation. The second is that the specimen can be maintained during transport in the same condition as when first collected, if suspended in a non-nutrient and non-inhibitory medium. So his outfit consists of a screw-capped bottle with a soft agar containing sodium thioglycollate to reduce oxidation, and another tube with sterile cotton swabs treated with charcoal to neutralize the bacteriostatic substances present in some of the commercial agars. The specimen is collected on the swab which is plunged immediately into this sloppy agar. The swab stick is snipped off with scissors and the cap on the bottle screwed on tightly. He found that this is an extremely useful method for transporting specimens for some distance. Furthermore, it is applicable not only to Salmonella and Shigella specimens but also to GC and meningococci specimens. This is something you might look into. The method was published in the March 1956 issue of the Canadian Journal of Public Health. Stuart's observations have been recently confirmed by Barlow (Jap. Jour. Med. Sci., 1955, **8**: 171), who suggested the use of cysteine, instead of thioglycollate to keep down the oxidation; it is less toxic.

With regard to the isolation of the pathogens, the effect of antibiotics has been mentioned. With antibiotics so widely used today, it is something you have to keep in mind. You cannot always get specimens collected before antibiotic treatment is started. Therefore, you must take the proper precautions mentioned. While antibiotics may interfere with the culture of the organism, they may also interfere with the appearance of the organism. They might so affect the morphology and staining characteristics, the cultural and/or the serological, that the typical characters by which we identify the pathogen may be so altered that we might fail to recognize it. Therefore, you should, in specimens that have come from antibiotic treated patients, be on the lookout for aberrant forms. This is something we know very little about. Also with regard to isolation techniques, there is another point of interest that I would like to mention here, namely, the isolation of *S. typhi* from the blood. Frequently, during stages of typhoid fever, blood culture is successful where fecal or urine cultures

are negative. Far too little blood culturing is done in P.U.O.'s. The introduction of antibiotic therapy has made it imperative that the diagnosis be achieved quickly. Watson and his co-workers in South Africa (Am. Jour. Trop. Med. Hyg., 1956, **5**: 131) have improved the method of isolating *S. typhi* from blood, a method which I would like to pass along to you as worthwhile. One of the objections to the use of blood culture has been that it takes too long to get results, particularly if it is a low-grade infection with which one is dealing. There are few organisms present initially in the specimen to grow, and the blood *serum*, very often, prolongs the incubation of the specimen for 4 to 5 days, sometimes for as long as 10 days, before a positive culture is obtained. This is not good enough today when prompt results are necessary to determine the right kind of antibiotic therapy to be used. Watson et al, by the use of blood *clots*, rather than whole blood, eliminates much of the bacteriostatic effect of the serum, and by culturing in a bile (0.5% sodium taurocholate) broth, containing 100 units per ml of Streptokinase, it is possible in most cases now, unless it is an extremely low grade infection, to get positive cultures within 24 hours. Here again is another technique that I think will well bear investigation.

The identification of the pathogens has been well covered by Dr. Kauffman and Dr. Edwards. All the new techniques, such as the KCN, ninhydrin and organic acids tests, etc., will help speed up the identification of the pathogen, will eliminate a lot of trouble and will also save money. In regard to sera, as Dr. Bailey has mentioned, here in Canada, we prepare at the Laboratory of Hygiene, for distribution to the Provincial Public Health Laboratories, diagnostic sera for most of the Salmonella and for the Shigella group. We cannot make these available to the hospital laboratories. But there are diagnostic sera available commercially, which will permit the hospital laboratory worker to identify most (over 90%) of the Salmonellae likely to be found in this country or in the United States. Shigella grouping sera and Alkalescens-Dispar Sera are also available commercially. We have heard today, several references to enteropathognic coli serotypes. Dr. Bailey mentioned that we produce polyvalent sera for the commonly reported pathogenic coli types, but we have again to restrict distribution to the Public Health Laboratories. The place for these coli sera is primarily the hospital laboratory. There are no special cultural tests which will distinguish the pathogenic from the non-pathogenic *E. coli* types. How then is the hospital laboratory technician, without these diagnostic sera, to know whether he is dealing with a special pathogenic type or with only the normal *E. coli* inhabitant of the gut? I am sure there is a considerably greater amount of 'coli-diarrhea' in this country and in the U. S. than we have any suspicion of, or than the medical profession has suspicion of, simply because the hospital laboratories have not been in a position to recognize it.

It is not good enough to have these sera located in the provincial or state public health laboratory, since these laboratories simply will never see the cultures if they are not 'spotted' in the hospital. The hospital technician holds a key position in recognizing this infection in the hospital. I am informed that there are at least two commercial houses which now have for sale diagnostic sera for some of these special *E. coli* serotypes. No good clinical bacteriological laboratory should be without them.

My congratulations to the Speakers for their excellent addresses.

AMONG THE NEW BOOKS

CLINICAL PATHOLOGY: Second Ed. By Benjamin B. Wells, M.D., Ph.D., Director of Clinical Investigation, The Lynn Clinic, Detroit. Former Professor of Medicine, Creighton Univ. School of Medicine, Omaha. W. B. Saunders Co. Philadelphia and London. 1956. 488 pp. 25 figures. 32 tables.

The author offers this volume primarily to medical students and physicians as a guide to clinical application and interpretation of laboratory studies. The first nine chapters are devoted to specific medical topics, each with brief discussions of the types of laboratory work required. The medical technologist will find much here to add interest to his efforts. These interpretations give the significance and reasons for performance of the tests noted. The final chapter with its short outlines of laboratory procedures would be necessary if there were not so many books available on techniques. However, although the technologist will find this of small use, the preceding chapters add considerably to the value of the volume.

HANDBOOK OF HISTOLOGY. Fourth Ed. By Karl A. Stiles, M.S., Ph.D., Professor and Head of the Department of Zoology, Michigan State University, East Lansing, Mich. The Blakiston Division, McGraw-Hill Book Company, Inc., New York, 1956. 240 pp. 10 tables. 7 figures. $3.00.

This was formerly the "Handbook of Microscopic Characteristics of Tissues and Organs." Morphology of the various tissues in outline style, with the tabular summaries, make this a very useful teaching adjunct to the histology laboratory. The student will find it well worth his while to know the types of tissue with which he is working.

ESSENTIALS OF HISTOLOGY. Third Ed. By Margaret M. Hoskins, Ph.D., and Gerrit Bevelander, Ph.D., New York University. 1956. The C. V. Mosby Company. St. Louis. 254 pages. 146 text illustrations. 2 color plates. 7 tables. $4.00.

The teaching essentials presented in this book will be of value to the histology student. There are numerous illustrations which will augment the narrative descriptions of the various tissues.

A LABORATORY GUIDE IN VIROLOGY. By Charles H. Cunningham, B.S., M.S., D.V.M., Ph.D., Professor of Microbiology and Public Health, Michigan State University, East Lansing, Mich. Burgess Publishing Co., Minneapolis, Minn. 1956. 145 pp. 10 figures. 31 tables.

As its name implies, this is a laboratory manual, with 44 laboratory exercises, a classification of Rickettsiae and Viruses, references, an appendix with sources of supply and one of logarithms.

DICTIONARY OF POISONS. By Ibert and Eleanor Mellan. Philosophical Library. New York, N. Y. 1956. 150 pages. $4.75.

Written in simple language, with topics listed alphabetically, this volume will be useful not only to the laboratory but in the home, or wherever there might be access to any of these poisons. Emergency treatment is discussed, including the removal of ingested poisons, preparation of antidotes. The different types of poisoning are given, with symptoms, antidotes, and first aid treatment.

Other Books Received:

EDUCATING SPASTIC CHILDREN. By F. Eleanor Schonell, M.A., Ph.D., Formerly Research Fellow, University of Birmingham, England. Department of Paediatrics and Child Health. Philosophical Library. New York, N. Y. 1956. 242 pp. 11 plates. 23 tables. 5 diagrams. $6.00.

SLEEP. By Dr. Marie Stopes. Philosophical Library, Inc., New York, N. Y. 1956. 154 pp. $3.00.

SYMPOSIUM: Electrophoresis.

ELECTROPHORESIS IN THE CLINICAL LABORATORY*

ANTOINETTE M. CALDARELLA, B.S., M.T. (ASCP)
St. Francis Hospital, Hartford, Conn.

Protein electrophoresis, that is, the separation of protein fractions by means of an electric current, is certainly not a recently discovered phenomenon. In 1930 Arne Wilhelm Kaurin Tiselius of Stockholm submitted a comprehensive review of electrophoresis as his thesis for a doctor of science degree. In 1936 he developed the moving boundary apparatus which has since been commonly called "The Tiselius Apparatus."[1] But his moving boundary procedure, since it requires the use of elaborate and costly equipment, is hardly suited to the means or abilities of a clinical laboratory—although it is precise and recognized as a standard for the study of proteins and other complex substances.

Within the past five years a method of zone electrophoresis has been developed. This is a technically simpler process which uses paper suspended in a buffered electroylte solution. The sample is placed at an appropriate point on the saturated paper strip, and when an electric current is passed through the paper, the various components of the sample will migrate at specific speeds exactly as in boundary electrophoresis. The obvious advantage is that the sample and its components are at all times captive on the paper and are therefore visible or recoverable at will. This process not only requires simple instrumentation, but it tremendously simplifies quantitation of the separated components by either visualization of their stained densities or by actual elution from the various segments of paper as is done in chromatography.

Although both the Tiselius and paper strip methods of protein partition have received extensive use as research and investigative tools in large centers, their use has not been very widespread in the general hospital clinical laboratory. The purpose of this report is to outline briefly how this type of study can contribute to patient care and diagnosis and to illustrate the usefulness of the procedure in our experience during the year 1955.

The Spinco Model R apparatus for filter paper electrophoresis has been used in all our determinations. Whatman #3 filter paper strips, measuring 30 cm. in length and 3.0 cm. in width, are vertically suspended in a Durrum-type cell containing a veronal buffer with an ionic strength of 0.075 and a pH of 8.6. The sample is placed on the wet strips after they have been equilibrated in the closed chamber. A constant current of 7.5

* First Award—Scientific Products Foundation, Biochemistry, 1956. Read before First North American Conference of Medical Laboratory Technologists, Quebec, Canada, June, 1956.

milliamperes is applied for a period of 16 hours. The strips are dried in a hot oven (120°-130°C) and stained with bromphenol blue.[2]

We have quantitated all our protein strips with the Spinco scanner integrator (Analytrol) in order to have numerical data for statistical analyses and comparative studies. However, for practical purposes quantitation is not absolutely necessary except that it allows for a numerical record on the hospital chart. The stained paper strips may be cut, and each protein fraction eluted in NaOH; the optical density of the eluted fraction may be then read on any spectrophotometer. Often visual comparison of the depth of color of each fraction with a normal pattern is sufficient to detect clinically significant changes in quantity.

The proteins which are separated from serum are albumin; alpha$_1$, alpha$_2$ beta and gamma globulins; and myeloma globulin when present. In our experience there is only one disease in which serum protein electrophoresis is diagnostic—plasma cell myeloma. The myeloma, or "M," band is easily recognized. It is a narrow, dense band occurring in the gamma position or between the beta and gamma globulins. It is distinct enough so that when superimposed upon gamma globulin the two can be readily distinguished, since gamma globulin has a more diffuse picture. This band is present only in cases of disseminated myeloma; to date no cases of the disease have been found in which the myeloma protein was not present. Whereas symptoms and histologic changes do not appear until myeloma is widespread and far advanced, "M" protein probably appears early in the course of the disease thus giving electrophoresis an advantage over other methods of diagnosis.[3]

The statistics alone on the incidence of myeloma in our hospital attest to the value of the electrophoresis apparatus in the early discovery of this disease. In 1955 a diagnosis of plasma cell myeloma was made in sixteen patients, twelve of whom were previously unsuspected clinically of having the disease, the diagnosis becoming clear as a result of the electrophoretic pattern done on them as problem cases. All diagnoses were confirmed by bone marrow study. In contrast to the sixteen cases diagnosed in the year 1955 is the record of the twenty years from 1935-1954, during which time a total of only seventeen cases were diagnosed.[4]

Paper electrophoresis has also made possible the detection of two cases of aggammaglobulinemia. In both patients the discovery of the absence of this protein produced the answer to the clinical problem of repeated severe infections. This represents another area of real usefulness for the procedure.

A third field of importance where protein fractions can be of real diagnostic and prognostic help is in the differentiation of

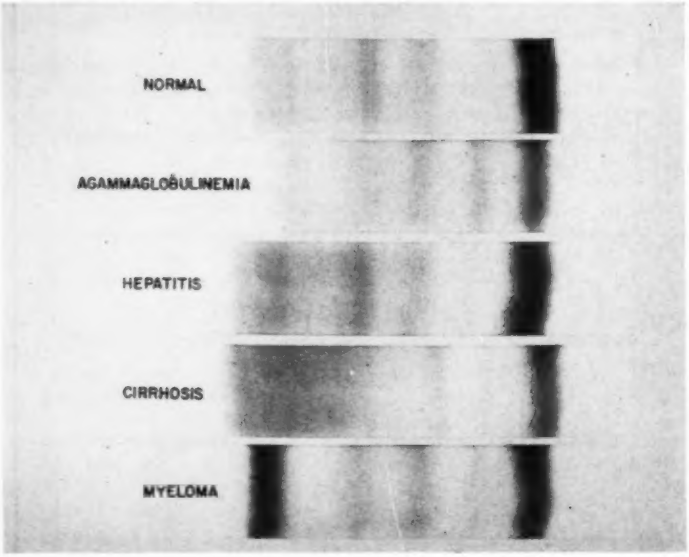


FIGURE 1

Here we have one normal protein pattern and four abnormal patterns. The albumin band is the farthest band on the right, the alpha, band is next to it, then come alpha$_2$, beta, and gamma globulins. The second strip illustrates an almost complete absence of gamma globulin. The third strip illustrates an increase in gamma globulin. In the cirrhosis patient notice the diffusion of beta and gamma globulins. The last strip illustrates a myeloma band.

jaundiced patients. In hepatocellular disease a distinct elevation of gamma globulin is readily apparent while in obstructive jaundice the pattern is normal. This has proven to be one of the most reliable of the liver function tests.[5]

Except for the above-mentioned cases, protein patterns in disease are non-specific. It is not within the limitations of this paper to list the great number of diseases which are associated with any increases or decreases in the five protein fractions; these changes and their significance have already been reported adequately in the medical literature.[6] The protein patterns which we have obtained on problem cases have aided the clinician a great deal by suggesting probable diagnoses and eliminating those inconsistent with the pattern obtained. For instance, the chemical A/G ratio may show an increase in globulin fraction, but it does not indicate which of the globulins is elevated; if

the electrophoretic strip shows an increase in gamma globulin rather than alpha$_2$, the implication is quite different than if the reverse were true. Thus, an abnormal pattern, though it is nonspecific, can give a clue to the clinician as to what possible diseases his patient may have.

This same technique and relatively simple apparatus can also be used for the differentiation of hemoglobins. Since the only value of hemoglobin electrophoresis is to identify the type of hemoglobin present, our determinations have been qualitative rather than quantitative. It is possible to differentiate between normal hemoglobin and most of the abnormal types.[7, 8] Our experience has included hemoglobin A (normal adult hemoglobin) and hemoglobin S (sickle hemoglobin). We have been able to differentiate between sickle cell disease and sickle cell trait in three cases of sicklemia. The same differentiation of trait from disease is possible in thalassemia which we have not as yet encountered.

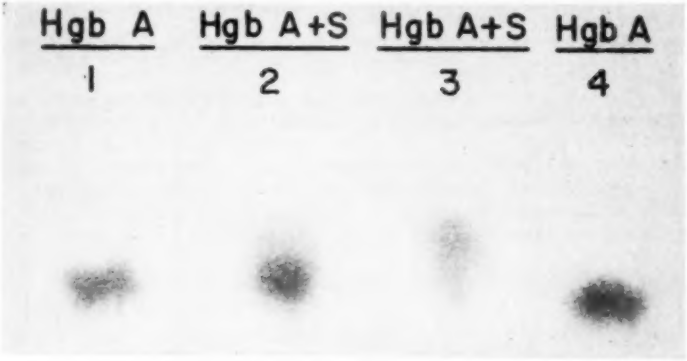


FIGURE 2

This illustrates Adult Hgb A and Sickle Hgb S. Notice that in the second illustrations there is approximately 60% Hgb A and 40% Hgb S. This is typical of sickle cell trait. In the third illustration the S hgb is approximately 90% as is found in sickle cell disease.

In this brief discussion of our experience with protein and hemoglobin electrophoresis, I have presented to you a few of the instances in which paper electrophoresis has aided in making a diagnosis. I have picked only the most striking examples for illustration. They by no means cover every case in which paper electrophoresis has aided the clinician. As a result of our experience with paper electrophoresis, we feel that this method has a definite place in the clinical laboratory and in the future may well prove to be of routine necessity.

BIBLIOGRAPHY

1. Gray, George W., "Electrophoresis," *Scientific American,* Vol. 185, p. 45-53, Dec., 1951.
2. Block, R. J., E. L. Durrum and G. Zweig, *A Manual of Paper Chromatography and Paper Electrophoresis,* New York, Academic Press, Inc., 1955.
3. O'Conor, G. T., and A. Caldarella, "Serum Electrophoresis, A Simple Test for Myeloma," in press.
4. ———————, *Ibid.*
5. Satokar, R. S., R. A. Lewis, B. B. Gaitonde, "Electrophoretic Studies of the Plasma Proteins in Virus Hepatitis," *J. Laboratory and Clinical Medicine,* Vol. 44, p. 349, 1954.
   Murray, Franklin, William B. Bean, William D. Paul, J. J. Routh, Jesus de la Huerga and Hans Popper, "Electrophoretic Studies in Liver Disease," Parts I and II, *Journal of Clin. Invest.,* Vol. 30, p. 718, 1951.
6. MacKay, Ian R., and Wade Volwiler, "Quantitative Aspects of Filter Paper Electrophoresis of Serum," *Australasian Annals of Medicine,* Vol. 3, No. 4, p. 263-269, Nov., 1954.
   Queen, E. G., R. F. O'Shea and the late M. P. Summerfeld, "Filter Paper Electrophoresis of Serum Proteins in Clinical Practice," *Australasian Annals of Medicine,* Vol. 3, p. 270, 1954.
   Kuhns, William J., "Abnormal Protein Patterns in Disease," *American Practitioner Digest of Treatment,* Vol. 6, p. 1157, 1955.
7. Motulsky, Anro G., Milton H. Paul and E. L. Durrum, "Paper Electrophoresis of Abnormal Hemoglobins and Its Clinical Applications, etc.," *Blood,* Vol. 9, p. 897, 1954.
8. Chernoff, Amoz J., "The Human Hemoglobins in Health and Disease," *New England J. of Medicine,* Vol. 253, p. 322, 1955.

ELECTROPHORETIC STUDIES OF SICKLE CELL ANEMIA*

ELEANOR E. STEPHENSON
620 E. Bluff, Apt. D, Fort Worth, Texas

Electrophoresis is that phenomenon in which charged particles in suspension or solution tend to migrate under the influence of an electrical potential. The direction and velocity of this migration depends upon certain characteristics of the particle itself, including its net charge and size, and factors attributable to the character of the electrical potential and buffer solution employed. The latter group of variables may be experimentally controlled so that it becomes possible to separate various fractions of a composite substance, provided that the fractions in question have different qualities of migration under a given environment.

This paper deals with the electrophoretic separation of types of hemoglobin associated with sickle cell anemia.

To obtain migration in an electrical field it is imperative that a particle possess a charge. Proteins are amphoteric substances; that is, they may act as acids or bases or remain completely neutral depending upon the pH of the solution to which they are exposed. The iso-electric point of a protein is the pH at which the protein exists in a neutral state. In such an environment no migration can occur. Due to the interaction of the amine group, which may become positively charged, and the carboxyl group, which may become negatively charged, a protein, when placed in a pH acid to the iso-electric point, will exist as a positively charged ion. Conversely, exposure to solution of pH alkaline to the iso-electric point produces a negatively charged ion.

The iso-electric points of some of the proteins found in human blood are as follows:[1]

Hemoglobin	pH 6.8
Serum Albumen	pH 4.7
Serum Globulin	pH 5.5

The barbital buffer solution used has a pH of 8.6 which is definitely basic to all these proteins. Therefore, the proteins found in this solution in a colloidal sol state will exist as bases, and will possess a negative charge. These negatively charged particles will migrate to the positive pole of the electrical field with a velocity determined by their individual mobility rates.

Methods and Materials

Hemoglobin solutions were prepared by a modification of

* Claudia M. Stannard Award. Read before First North American Conference of Medical Laboratory Technologists, Quebec, Canada, June, 1956.

method suggested by Drabkin.[2] Erythrocytes obtained from oxalated blood were washed four times with 0.9% sodium chloride. These washed cells were then hemolyzed with an equal volume of distilled water, and 0.4 volumes of toluene were added for the removal of lipids. This mixture was centrifuged for 20 minutes at 3000 RPM. The upper two layers were removed and the remaining hemoglobin concentrate diluted to approximately 3%. Then, 0.02 cc. of this solution were streaked on strips of filter paper which were arranged on the electrophoretic apparatus.

The appratus consisted of a divided buffer container in which the electrodes were placed. The strips of filter paper were set between glass plates, which were supported above the chamber by a platform. A heavy cloth strip was stretched the length of each chamber and partially immersed in the buffer solution. The ends of the filter papers were attached to these cloths rather than being placed directly in the liquid.

Veronal barbital buffer of pH 8.6, ionic strength 0.05 was used. This particular pH has been found to be the most satisfactory reaction for clear separation of the hemoglobin fractions, since each component has a distinctly individual mobility rate at this pH. An electrical potential of 280 volts was applied for 4½ hours.

The staining procedure employed a 0.75% alcoholic solution azo-carmine G, in which the strips were immersed for six minutes. The strips were than placed for six minutes in a mordant solution composed of 400 cc. of absolute methanol, 100 cc. of glacial acetic acid, and 500 cc. of distilled water. The decolorizer used consisted of 10% glacial acetic acid.

Results

In a small percentage of the Negro population, there has been observed a tendency of erythrocytes to assume bizarre shapes under certain conditions.[3] These cells when exposed to a low oxygen tension tend to sickle in varying degrees, due to an hereditary malfunction of the bone marrow which produces potentially deformed cells.[4]

The gene transmitting this inherited tendency when present in an heterozygous state results in sicklemia or sickle cell trait, a condition seldom of pathological significance. However, in the homozygous condition this gene produces sickle cell anemia, a severe, chronic hemolytic anemia.[3]

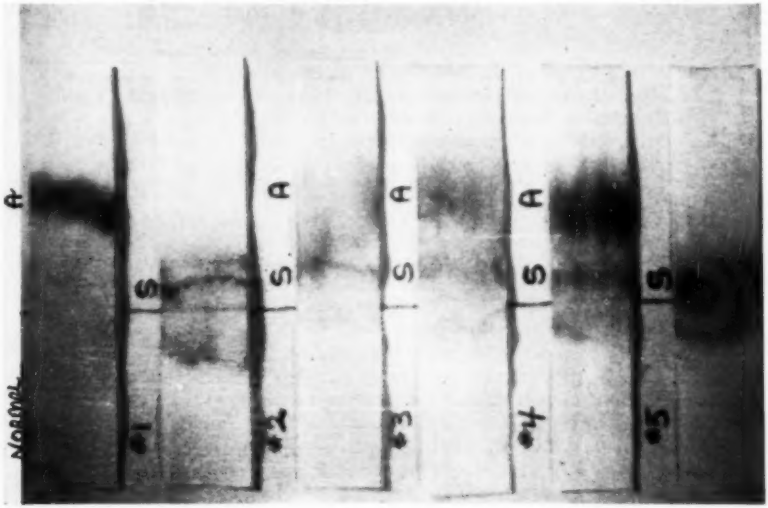
Hemoglobin preparations from five known sickle cell subjects were electrophoretically separated. Subjects 1 and 5 are sisters, and subjects 2, 3 and 4 are daughters of subject 1.

The following chart is presented showing comparative hematological studies of each subject:

	Red Count in Millions/cu. mm.	Appearance of Direct Smear	Hemoglobin in Grams/100 cc.	Percentage Sickling in Sickle Cell Preparation*
Normal Subject	4.5	Normal	13.0 gm.	0%
Subject # 1	4.6	Normal	13.2 gm.	95%
Subject # 2	2.7	Marked hypochromia, moderate poikilocytosis and anisocytosis	7.9 gm.	45%
Subject # 3	3.4	Moderate anisocytosis and poikilocytosis	9.3 gm.	35%
Subject # 4	4.0	Moderate hypochromia and marked anisocytosis	11.1 gm.	35%
Subject # 5	4.0	Normal	11.8 gm.	95%

Electrophoretic techniques have separated normal hemoglobin, designated as hemoglobin A, from the abnormal hemoglobin associated with sickle cell anemia, hemoglobin S. Both fractions were found to migrate to the anode; however, hemoglobin A migrates with the greater velocity.[5]

The photograph shows the electrophoretic hemoglobin migration patterns of each subject compared to normal control pattern.



* Sodium metabisulfite preparations.

Patterns of subjects 2, 3 and 4 indicate the presence of hemoglobin A, and hemoglobin S. Patterns of subjects 1 and 5, however, indicate the hemoglobin of these individuals is composed almost entirely of hemoglobin S. These findings are in agreement with the percentages of sickle cells observed in the sodium metabisulfite preparations.

The migration patterns of subjects 2, 3 and 4 parallel the characteristic pattern of sickle trait noted by Motulsky, Paul and Durrum,[5] while the patterns of patients 1 and 5 closely resemble the typical pattern associated with true sickle cell anemia. The clinical diagnosis made by the physician of these subjects supports this observation.

Although the father of subjects 2, 3 and 4 was not available for sickle cell studies at this time, he has never exhibited clinical symptoms of the disease. Assuming that he is homozygous for normal hemoglobin characteristics, the mating of this individual with subject 1, who appears to be homozygous for sickle anemia, has produced three children having the heterozygous sickle cell trait.

Summary

With the electrophoretic technique employed, it has been possible to separate hemoglobin A from hemoglobin S, and to differentiate true sickle cell anemia from the sickling trait. The results obtained are confirmed by other laboratory findings and by the clinical diagnoses.

REFERENCES

1. Hawk, Phillip B., and Bergeim, Olaf: *Practical Physiological Chemistry*, Blakiston's Son & Co., Inc., Philadelphia, pp. 126, 1931.
2. Drabkin, David L.: "Spectrophotometric Studies," *J. Biol. Chem.*, 164: 703-723, 1946.
3. Neel, James V.: "Inheritance of Sickle Cell Anemia," *Science*, 110: 64-66, 1949.
4. Blackfan, Kenneth D., Diamont, Louis K., and Leister, Merrill C.: *Atlas of the Blood in Children*, Commonwealth Fund, New York, pp. 83-86, 1944.
5. Motulsky, Arno G., Paul, Milton H., and Durrum, E. L.: "Paper Electrophoresis of Abnormal Hemoglobins," *Blood*, 9: 897-910, 1954.

PAPER ELECTROPHORESIS—GENERAL PRINCIPLES

KATHRYN CATALANO
501 Garfield, Denver, Colo.

Electrophoresis depends upon the ability of electrically charged particles or molecules in a solution or suspension to migrate in an electrical field. The velocity is determined by the charge and the properties of the different materials being determined. When ions having different properties migrate at different rates, they can be separated by electrophoresis.

The "classical" apparatus for electrophoresis was devised by Tiselius and consists of a glass U-tube composed of cells with parallel walls of optic glass connected through columns of liquid to the negative and positive electrodes. The ability to get a picture of the separated protein fractions is based on the fact that there is a refractive index difference between the buffer solvent and the protein solution. Gronwall (1), in order to remove the convection currents and stabilize the moving boundary separation, has performed the electrophoresis of protein mixtures and amino acids in packed cotton, glass wool, asbestos, silica gel and agar.

The principle of paper electrophoresis is essentially the same except that the migration occurs along a filter paper which has been moistened with a buffer solution and placed between two electrodes. This filter paper is placed in a wet chamber which is to eliminate evaporation from the paper.

The actual methods used vary somewhat but are all basically the same. For example, the sample to be tested is applied to a strip of filter paper which has been moistened with the electrolyte buffer. The ends of this paper are connected through reservoirs of electroylte, usually also covered by heavy paper wicks, to the electrodes which consist mainly of platinum wire. In most methods these strips are supported in an inverted V. The sample is applied to the vertex of the V and allowed a short while to be absorbed. In some methods the sample is applied before moistening the filter paper, in others the paper is dipped in the electrolyte first. The circuit is then completed and is allowed to run about 16 hours. The buffer may consist of varying amounts of diethyl barbituric acid and sodium diethyl barbiturate or veronal. After running the prescribed length of time, the papers are dried in ovens varying from 120-140 degrees Centigrade. They should not receive the direct radiation and should be out of drafts. Dry for about 30 minutes to insure the coagulation of the protein, then place in a stain for about six hours. The dye is made up of bromphenol blue, zinc sulphate, and 5% acetic acid. The papers are then rinsed in 5% acetic acid twice, placed

in a fixative consisting of sodium acetate and 5% acetic acid, and finally dried. The amount of dye which is bound is an index of the amount of protein in different parts of the paper. By cutting the paper and removing the dye and then colorimetrically determining the dye, a curve similar to the curve plotted in the "classical" Tiselius method is obtained by plotting the dye concentration against the distance traveled. The dye can also be photometrically determined without eluting the dye.

Gronwall (1) worked with the processes which occur during the electrophoresis when ions and charged protein molecules migrate, diffusion occurs, heat is generated which increases evaporation from the surface, and a hydrodynamic equilibrium is formed between the capillary force and the force of gravity in the minute pores of the paper. For best results a constant migration must take place thus necessitating a constant concentration and composition of the electrolyte which is altered by evaporation and the reaction at the electrodes. In order to keep all the deterent forces at a minimum a barbital buffer of pH 8.6-8.8, of ionic strength of 0.05, using a 35 cm. long strip of filter paper and a potential gradient of about 7-10 volts/cm. must be used to get a complete separation of 0.005-0.03 ml. of serum in about five hours. Gronwall (1) also recommends not only bromphenol blue, but also azocarmine S and amidoblack 10B. In most methods the dye is eluted with precisely measured amounts of dilute NaOH. The methods vary in whether the paper is cut in 5 mm. strips, or whether the entire segment of a single protein is cut.

Much work has been done checking the accuracy of this method against the "classical" method of Tiselius. Levin and Oberholzer (2) found that after delineating the protein fractions on paper, a micro-Kjeldahl method of determining the protein nitrogen gave better comparisons than the dye eluting method used by most. This is due to the fact that albumin has a greater binding capacity for the bromphenol blue than the gamma globulin. They found that from their graph 1.3 was a sufficient correction factor for the gamma portion. This would not apply to all fractions and is also specific to the time and concentration of the dye because of differential rates of adsorption. They also feel that the curve made by the dye elution method is difficult and not very accurate because of the job of measuring the 5 mm. strips of paper, the number of solutions on which the dye must be determined, and the extreme difficulty of analysing the curve once it is made. Levin and Oberholzer (2) ran a test to find out if the fractions were being correctly defined at the lowest point of concentration between the fractions. In one set the dye was eluted in 5 mm. strips and micro-Kjeldahls were done on another set. The latter was discovered to be as accurate in the

method of compiling and analysing the curve as the Tiselius method. Comparison of the two methods showed that the albumin fraction agrees quite well, but the gamma globulin, showed several differences over 6%. The greatest difference (over 10%) was shown on a patient with multiple myeloma. However, the gamma globulin, being an end fraction like albumin, should be expected to agree. The discrepancy may come because of extra adsorption at the point of application. Methods done on nephrotic serum showed difficulty in separating the $alpha_2$ and beta globulin fractions partly due to the large amount of lipids. They also do not agree too well on the determination of hyperglobulinemia, possibly because of the dye elution method giving a measure of the protein part of the protein-lipid-carbohydrate complex. In comparing salt fractionation and paper methods, it was found that the two methods correlated well except in the case of nephrosis and in the $alpha_2$ and beta fractions. In salt fractionation most of the globulin appears in the beta fraction, in the paper the globulin is mainly in the $alpha_2$.

Hardwicke (3), in a paper covering different determinations made to check the accuracy of paper electrophoresis against the "classical" Tiselius, says that the quantitative accuracy is found to be plus or minus 6% of the total protein present. He feels that this method may be quantitatively more accurate in the analysis of pathological sera containing high proportions of lipid or carbohydrate than any process used up to now.

Paper electrophoresis can also be used for spinal fluid. Before use, it is concentrated by dialysis in a cellophane bag against a concentrated solution of dextran. About nine ml. of spinal fluid of normal protein content can be concentrated to about 0.2 ml. after approximately 24 hours of dialysis in a cold room. After being concentrated spinal fluid will show a paper pattern similar to normal serum. Gronwall (1) worked out a method for the staining of serum-bound fat after the regular period of electrophoresis. After staining with a half saturated Sudan black in 50% ethyl alcohol, the fat will appear as blue-black spots situated in the globulin fractions. Glyco and muco protein may be stained by a modification of the Hotchkiss method, using periodic acid for the oxidation of polysaccharides to polyaldehydes. The protein polysaccharides will appear on the paper with a red-violet color.

Paper electrophoresis is also very useful in separating and analysing abnormal hemoglobins. Spaet (4) states that Pauling and his associates discovered that the sickling of red cells is associated with an abnormal hemoglobin of altered electrophoretic mobility. Later Itano and others found a second type having an electrophoretic mobility different from normal and sickle hemoglobins. When this second type is combined with normal

blood target cells are seen in peripheral blood smears although there is no clinical evidence of disease. This is designated as hemoglobin C. Itano also found another hemoglobin which has mobility of the same magnitude as sickle, but is more soluble and is not capable of causing the erythrocyte to sickle. It is also true that adult and fetal hemoglobins differ in their electrophoretic mobility.

In order to prepare bloods for electrophoresis the cells must first be hemolysed. Spaet (4) used oxalated blood, washed the cells three times with normal saline, hemolysed the packed cells by freezing, then thawing. He then diluted the resulting solution with five volumes of veronal buffer (pH 8.6) and finally centrifuged an hour at 20,000 r.p.m. Another method washes the cells with 0.9% NaCl, adds an equal volume distilled water to the packed cells. Using normal hemoglobin as a control, Spaet (4) showed that normal hemoglobin travels farthest, sickle intermediate, and hemoglobin C the least. Motulsky, Milton and Durrum (5) showed this to be true and in addition they showed that fetal hemoglobin is between normal and sickle types.

Motulsky, Milton and Durrum (5) state that paper electrophoresis may be used for life span determinations of the erythrocytes in some of the hereditary hemoglobinopathies. The decay of normal adult hemoglobin from transfused normal cells may be followed in patients with homozygous hemoglobin C red cells by following the disappearance on paper. Conversely, homozygous hemoglobin S or homozygous hemoglobin C could be given to a recipient with homozygous hemoglobin A cells and the disappearance curve of the abnormal fraction followed.

In the past paper electrophoresis has been mainly a tool of research. Now it is becoming a very popular diagnostic tool. It is a practical method because of the many determinations which can be done, the small amount of specimen needed, the many specimens which can be run simultaneously, the simplicity of procedure, its ready availability, and perhaps most important, its future promise to make the analysis of colored solutions and turbid solutions easier.

REFERENCES

1. Gronwall, A., "On Paper Electrophoresis in the Clinical Laboratory," Scandinavian J. of Clin. and Lab. Investigation, **4,** 270-279, 1952.
2. Levin, B., and V. Oberholzer, "Paper Electrophoresis of Serum Protein," Am. J. Clin. Path., **23,** 205-216, 1953.
3. Hardwicke, J., "The Estimation of Serum Proteins by Electrophoresis on Filter Paper," Biochemical Journal, **57,** 166-171, 1954.
4. Spaet, Theodore H., "Identification of Abnormal Hemoglobins by Means of Paper Electrophoresis," Journal of Laboratory and Clinical Medicine, **41,** 161-165, 1953.
5. Motulsky, A. G., H. P. Milton and E. G. Durrum, "Paper Electrophoresis of Abnormal Hemoglobins and Its Clinical Applications," Blood, **9,** 897, 1954.

AN ELECTRICALLY HEATED AND THERMOSTATICALLY CONTROLLED OVEN FOR DRYING ELECTROPHORESIS PAPER STRIPS*

CHARLES D. BROWN
Albany, N. Y.

This inexpensive oven for drying filter paper used for electrophoretic tracings was constructed in the Division's Sheet Metal Shop and the electrical parts were installed by the Laboratory Electrician. The apparatus is superior to an electric hot plate which has an inadequate temperature control, and obviates the use of a more expensive laboratory oven. (Fig. 1.)

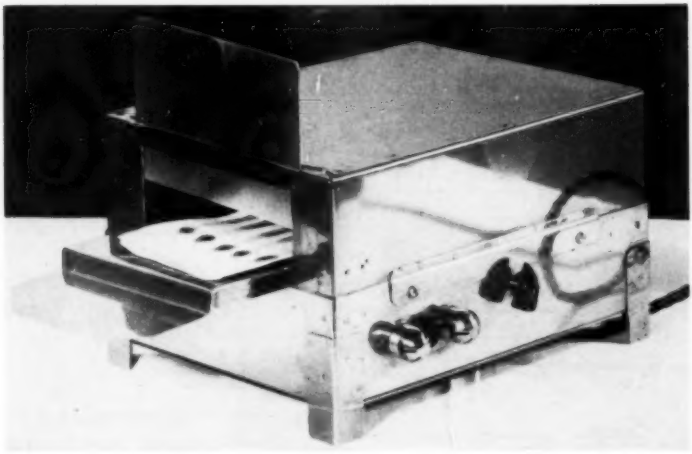


FIGURE 1
Oven for drying electrophoresis paper strips.

Description of apparatus. Construction is of stainless steel sheet metal 24 gauge and ¼″ aluminum plate (Fig. 2). Over-all dimensions are 9″ by 12″ by 6¼″ high. The upper half of the oven is demountable by removing 4 sheet metal screws which anchor the top section to the bottom containing the electrical units. The bottom plate is removable by the same procedure to allow access to the electric parts. All other metal parts are fabricated by electric spot welding. The heater unit is a 200-watt Chromalox heating strip regulated by an adjustable Chrom-

* From Division of Laboratories and Research, New York State Department of Health, Albany, New York.

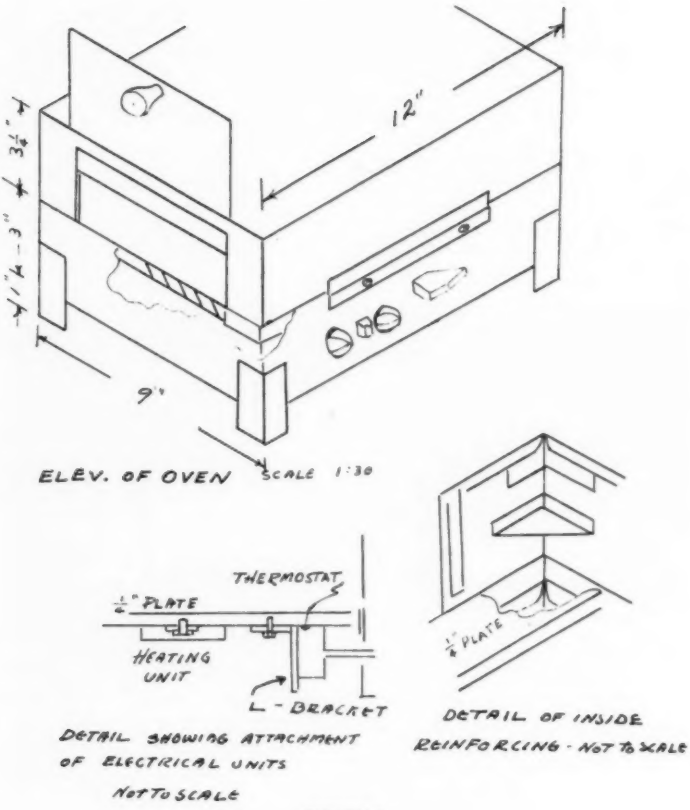


FIGURE 2
Drawing showing structural details.

alox clamp-on thermostat having a temperature range from 100°F. to 450°F. The thermostat setscrew adjusts the temperature range approximately 1°F. per degree of angular turn. Turning the setscrew clockwise from minimum temperature will furnish the desired temperature. Wiring of the heating unit and thermostat is shown in Figure 3. Two telltale lamps in parallel indicate, respectively, that the line current is on and that the heating unit is functioning. Opening 2½" by 6" in front of oven is closed by a sliding panel.

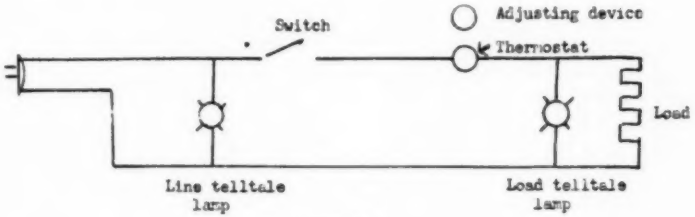


FIGURE 3
Wiring diagram.

Operation. The oven operates on 115V, AC, and requires only a few minutes to reach operating temperature of 221°F. (105°C). The filter paper to be dried is placed upon a removable palette 6″ by 14″ with ½″ 90° bend at each end to space it ½″ above heating plate. The palette is then placed in the oven, the oven panel being left open to allow the steam to dissipate; the panel is then closed and the drying process is completed. The top of the oven is used also for drying the developed paper tracings following dehydration in alcohol.

Summary

The oven described is inexpensive, easy to operate, has adequate temperature control, reaches temperature rapidly, and is in general satisfactory for drying electrophoresis paper strips.

Awards Made Available to Members of the American Society of Medical Technologists

THE KIMBLE ACHIEVEMENT AWARD

The Kimble Achievement Award is to be made for the first time in 1957. It consists of a $500.00 cash award, an engraved sterling silver plaque to serve as a permanent testimonial to the recipient(s), and travelling and hotel expense incurred during the convention. The candidate's achievement should be outstanding in the field of laboratory science. This can be a development of new techniques, a report of new interpretations of accepted methods which would enable the medical profession better to diagnose or treat disease, or an improvement of existing techniques. A candidate must be an active member of ASMT and must have maintained that status for at least five years prior to nomination.

His work must have been completed within the twelve months preceding February 1st of the judging year. It must be in manuscript form with a bibliography or a reprint of the manuscript if it has been published. Three copies of the manuscript or reprint must be submitted. Three copies of a **curriculum vitae** of the candidate must accompany the manuscripts.

This award is presented by the Kimble Glass Company of Toledo, Ohio.

All nominations for the 1957 award must be sent by February 1st to:

C. Patton Steele, Chairman, Board of Directors
American Society of Medical Technologists
Box 1020
Bismarck, North Dakota.

THE CORNING AWARD

The Corning Award of $500.00 and a Steuben glass bowl with appropriate inscription will be presented for 1957 to the outstanding "Line Technologist" by the Corning Glass Works of Corning, New York.

A member of the ASMT may not spend more than half of his time in administrative duties to be eligible for this award, and must be performing general laboratory work a portion of the time.

He should be outstanding in the field of medical technology as well as a good citizen of the U.S.A.

Information should be presented on education background; training in medical technology; experience in the field of medical technology; offices held in the state and local organizations; activities outside of medical technology such as church, civic groups; etc.; scientific and other papers written; teaching or professional ability shown; and any other information that would indicate that this person is an ideal candidate for the award.

Each constituent society of the American Society of Medical Technologists is allowed one nominee.

Full information **must** be sent at the same time that the name is submitted to the Public Relations Counsel no later than April 1, 1957.

Announcement of the award winner will be made by a representative of Corning Glass Works at the annual convention of ASMT.

For further information concerning either of these awards write ASMT, Suite 25, Hermann Professional Building, Houston 25, Texas.

DOES NOT CIRCULATE

UNIVERSITY OF MICHIGAN
JAN 7 1957
MEDICAL LIBRARY

The American Journal of Medical Technology

DECEMBER, 1956

Vol. 22, No. 6a

ENTERIC DISEASE SYMPOSIUM ISSUE

OFFICIAL PUBLICATION
© 1956
by

AMERICAN SOCIETY OF MEDICAL TECHNOLOGISTS

Published Bi-Monthly by The American Society of Medical Technologists

Printed by The Gulf Printing Company

Business and Editorial Office: Suite 25, Hermann Professional Bldg., Houston 25, Texas

Printing Office: 3301 Buffalo Drive, Houston 6, Texas

DIFCO

PPLO

ISOLATION and CULTIVATION

THE AVAILABILITY of media for the isolation and cultivation of *Pleuropneumonia-Like Organisms* (PPLO) has increased the interest in these infectious agents. PPLO are readily grown from clinic specimens or from pure culture on Bacto-PPLO Agar and Bacto-PPLO Broths enriched with Bacto-PPLO Serum Fraction or Bacto-Ascitic Fluid.

BACTO-PPLO AGAR is an especially prepared solid medium containing the essential factors from meat infusion required by the PPLO. This medium, enriched with Bacto-PPLO Serum Fraction, will support abundant growth of all PPLO strains.

BACTO-PPLO BROTH w/o CV enriched with Bacto-Ascitic Fluid or Bacto-PPLO Serum Fraction is a liquid medium for enrichment and cultivation of PPLO organisms. Selective media for PPLO may be prepared from Bacto-PPLO Broth w/o CV by the addition of thallium acetate, potassium tellurite, penicillin or other bacteriostatic agents as desired.

BACTO-PPLO BROTH with CV enriched with Bacto-Ascitic Fluid is a selective medium for the isolation and cultivation of PPLO from specimens contaminated with commensal organisms. Contains Crystal Violet for inhibition of Gram-positives. The selectivity of the medium may be further increased to inhibit Gram-negatives by the addition of Bacto-Chapman Tellurite Solution. This medium will support growth of all PPLO strains and effectively suppress the growth of most contaminating bacterial flora.

BACTO-PPLO SERUM FRACTION contains the growth accessory factors required by all strains of the PPLO and is used as an enrichment in Bacto-PPLO Agar and Bacto-PPLO Broth w/o CV.

BACTO-ASCITIC FLUID is a sterile, bile-free ascitic fluid specially prepared for enriching culture media used in the cultivation of the PPLO group and other fastidious microorganisms.

BACTO-CHAPMAN TELLURITE SOLUTION to be added to Bacto-PPLO Broth w/CV or Bacto-PPLO w/o CV for the inhibition of Gram-negative microorganisms.

THE DIFCO MANUAL, *NINTH EDITION*,
including descriptions of these media and their use,
is available on request.

DIFCO LABORATORIES
DETROIT 1, MICHIGAN

SOCIETIES CHARTERED BY THE A. S. M. T.

1. Texas Society of Medical Technologists
2. Kentucky State Society of Medical Technologists
3. Nebraska Society of Medical Technologists
4. Minnesota Society of Medical Technologists
5. Oklahoma Society of Medical Technologists
6. Colorado State Society of Medical Technologists
7. Indiana Society of Medical Technologists and Laboratory Technicians
8. Illinois Medical Technologists' Association
9. District of Columbia Society of Medical Technologists
10. Pennsylvania Society of Medical Technologists and Laboratory Technicians
11. Georgia Society of Medical Technologists
12. Wisconsin Association of Medical Technologists
13. Ohio Society of Medical Technologists
14. Empire State (N. Y.) Association of Medical Technologists
15. Arkansas Society of Medical Technologists
16. Louisiana State Society of Medical Technologists
17. Tennessee Association of Medical Technologists
18. Massachusetts Association of Medical Technologists
19. Michigan Society of Medical Technologists
20. New Hampshire Society of Medical Technologists
21. California Society of Medical Technologists
22. Virginia Society of Medical Technologists
23. Association of Oregon Medical Technologists
24. Utah Society of Medical Technologists
25. Mississippi State Society of Medical Technologists
26. Maryland Society of Medical Technologists
27. Missouri Society of Medical Technologists
28. Kansas Society of Medical Technologists
29. North Carolina Society of Medical Technologists
30. Delaware Society of Medical Technologists
31. Connecticut Society of Medical Technologists
32. New Mexico Society of Medical Technologists
33. South Dakota Society of Medical Technologists
34. North Dakota Society of Medical Technologists
35. Florida Division of A.S.M.T.
36. New Jersey Society of Medical Technologists
37. Washington State Society of Medical Technologists
38. West Virginia State Society of Medical Technologists
39. Wyoming Society of Medical Technologists
40. Alabama Society of Medical Technologists
41. Montana Society of Medical Technologists
42. Vermont Society of Medical Technologists
43. Idaho Society of Medical Technologists
44. Iowa Society of Medical Technologists
45. Hawaii Society of Medical Technologists
47. Rhode Island Society of Medical Technologists
48. South Carolina Society of Medical Technologists and Laboratory Technicians
49. Maine Association of Medical Technologists
50. Arizona Society of Medical Technologists
51. Alaska Society of Medical Technologists
52. Isthmian Society of Medical Technologists (Charter pending)
53. Puerto Rico Society of Medical Technologists (Charter pending)

TO CONTRIBUTORS AND CORRESPONDENTS OF THE JOURNAL

Published January, March, May, July, September, November
Editorial and Business Office: Suite 25, Hermann Professional Bldg., Houston 25, Texas

ORIGINAL ARTICLES

Brief reports of new and interesting methods and results pertaining to the laboratory field are solicited. None will be considered for publication except with the understanding that it becomes the property of the Society.

Manuscripts must be typewritten and double spaced. (Do not submit carbon copies.) All figures and tables must be in form for the printer.

ILLUSTRATIONS

Cuts must be supplied or can be arranged for by the Editor. Expenses defrayed by the author if in excess of one cut. Drawings must be on white paper in India ink.

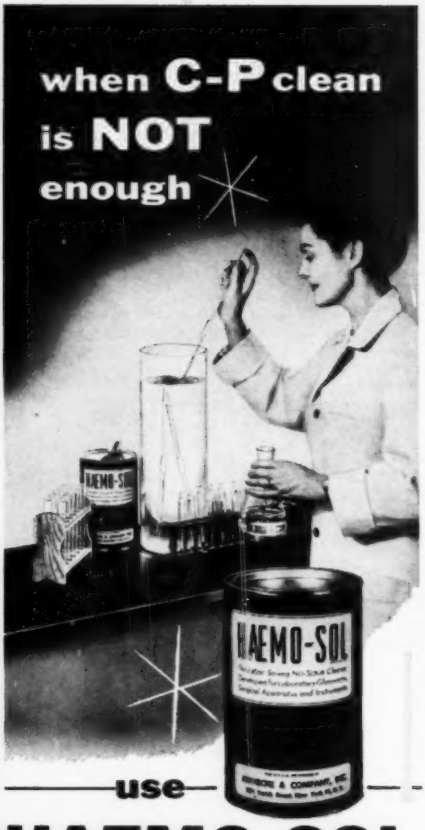
Reprints of original articles can be obtained. Order blank with price list is sent with galleys.

SUBSCRIPTION PRICE

$5.00 a year in United States, Canada and Countries within the postal union; $5.50 for countries outside the postal union; single copy, $1.00. The subscription of members of the American Society of Medical Technologists is included in the membership dues and is handled by the Executive Secretary of the Society. Other subscriptions should also be sent to the Executive Secretary, Suite 25, Hermann Professional Bldg., Houston 25, Texas.

Please notify Business Office of change of address

when C-P clean is NOT enough



use

HAEMO-SOL

100% effective in repeated use. Will not etch glass.

Prevent cleansing-related variables, save time with Haemo-sol; it strips tubes and vessels of proteins, oils, fats and all other soils, leaves laboratory glassware not merely C-P but absolutely clean. Haemo-sol is completely rinsable, completely soluble, has low surface tension and controlled pH. Because it leaves no residue, it is especially valuable in the most exacting titrations and tests. Cleanse with Haemo-sol for consistently accurate results—always.

Literature and samples on request.
Ask us about your special cleaning problem.

MEINECKE & COMPANY, Inc.
225 Varick Street, New York 14

Next Registry Examination

in

Medical Technology

TUESDAY
July 2, 1957

The July date is again being used as an experiment, giving the Board of Registry a complete cycle of one full year's statistics on the suitability of this time. The Board has set the examination on Tuesday because neither Wednesday, the day before the Fourth nor Friday, the day after, seemed appropriate.

Deadline for applications
May 1, 1957

REGISTRY OF MEDICAL TECHNOLOGISTS

of the
AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

P. O. Box 44
Muncie, Indiana

dd confidence
to your
cross match
Ortho
ANTI-HUMAN
SERUM
FOR THE COOMBS
ANTI-GLOBULIN TEST
Ortho Pharmaceutical Corporation
RARITAN, NEW JERSEY
Ortho

SERVALL® LABORATORY INSTRUMENTS

QUALITY · SERVICE · DEPENDABILITY

Backed by over 25 years of pioneering and engineering "know-how."

SS-1 SUPERSPEED ANGLE CENTRIFUGE

15,500 r.p.m.—31,000 × G—400 ml. Rotor accommodates eight 50 ml. tubes at 34° angle in separately numbered compartments. Accessory adapters available for 15 ml., 10 ml., or 5 ml. tubes.

Self-centering, no-thrust drive—ORGINATED BY SERVAL—promotes smoothness, rapid sedimentation, long bearing-life.

Type SS-1A: same as SS-1 but with water-cooled bearing assembly for extra-heavy duty. *Write for Bulletin JT-SIS.*

SMALL, MEDIUM, LARGE ANGLE CENTRIFUGES

Speeds range from 2,500 r.p.m. to 6,000 r.p.m. SERVAL Centrifuges' extreme versatility is demonstrated by varying quantities that may be spun, many rotors available, accommodation of adapters. *Write for Bulletin JT-SIM.*

REFRIGERATED SUPERSPEED CENTRIFUGES

For temperatures well below 0° C. Max. speed with 400 ml.: 16,000 r.p.m.—32,700 × G. Max. capacity at lower speeds: 2,000 ml. Direct drive. Advanced-design evaporator. Many other custom features. *Write for Bulletin JT-SIR.*

ULTRA-MICROTOMES 1/40—1/2 MICRON (PATENTS PENDING)

For Ultra-Thin, Uniform Sectioning as required by electron microscope. Collet-type chuck. Accurate mechanical advancement. Self-compensating suspension of all mechanical components. *Write for Bulletin JT-S5.*

HIGH-SPEED HOMOGENIZERS

For rapid, safe homogenising, mixing, disintegrating. 84,000 shearing cuts per min. Leak-proof construction. Shaped stainless-steel mixing chambers. 200 ml. capacity. *Write for Bulletin JT-S6.*

STERLING AUTOMATIC PIPETTES

For routine laboratory work in dispensing aqueous solutions. MODEL 4-3M: calibrated for delivery ranges 0.1 to 3.0 ml. MODEL 4-10: calibrated for deliveries 1-10 ml. *Write for Bulletin JT-S2.*

Ivan Sorvall, Inc.

NORWALK CONNECTICUT

This Coleman Team means better work — increased scope for the Clinical Laboratory

Lightens the laboratory work load . . . this team of Coleman Instruments not only lightens the present work load with faster, more dependable analyses . . . but prepares the laboratory for the even greater demands in hospital and laboratory facilities that come with expanding medical services.

Wider professional scope for the technologist . . . this reliable *proven* instrumentation brings greater confidence and speed . . . *plus* access to the many new and improved analytical technics developed by prominent authorities *on and for Coleman Instruments.**

The Coleman Junior Spectrophotometer stands throughout the world as the unquestioned choice of analytical instruments for the clinical laboratory. Fifteen years of dependable service—uncounted volumes of analytical methods stand behind this preference.

The Coleman Flame Photometer is the only instrument designed specifically for the direct measurement of Sodium, Potassium and Calcium in the biological samples encountered in the clinical laboratory.** Together, the Coleman Junior Spectrophotometer and the Coleman Flame Photometer provide the finest and most dependable of all analytical tools for clinical analysis—and at the lowest possible cost.

New Procedures.....

Coleman

* ☐ A Colorimetric Method for SGO Transaminase on the Coleman Junior Spectrophotometer—Bulletin T-143.

** ☐ A Rapid (Micro) Method for the determination of Sodium and Potassium in Serum or Plasma—Bulletin T-142.

☐ Detailed literature on Coleman Instruments.

NAME _____

TITLE _____

ORGANIZATION _____

ADDRESS _____

CITY _____ ZONE _____ STATE _____

Coleman Instruments Inc., Dept. M, 318 Madison St., Maywood, Ill.

Please Mention Publication When Writing Advertisers

Buy and use

Christmas Seals . . .

Fight Tuberculosis!

Laboratory Technologists—2 ASCP Registered or eligible. Pleasant suburban community near Chicago, Illinois. Pathologist supervision in well equipped general hospital laboratory. Min. starting salary $375-$400. Write ASMT Box No. 6H.

Medical Technologist (ASCP): 118 bed general hospital located in a beautiful residential suburb along the North Shore of Chicago. Modern ranch style homes with attractively furnished private bedrooms. Modern laboratory; full time pathologist. Salary $325.00. Paid call once a week. 40-hour week. Contact Personnel Office, Highland Park Hospital, Highland Park, Illinois.

Technologist: Blood gas analysis for Cardio Pulmonary Laboratory. Apply Personnel Lenox Hill Hospital, 111 East 76th Street, New York.

AN AUTHORIZED BINDING FOR THE AMERICAN JOURNAL OF MEDICAL TECHNOLOGY

Arrangements have been made for subscribers to have their journals bound into distinctively designed books.

These seven issues, January through December, bound in best grade sapphire washable buckram, imprinted with your name on cover, cost but $3.55 per volume.

Bound journals serve as an immediate reference for research and information. Properly displayed, they imply the time and effort spent to keep up-to-date on the most modern techniques.

Ship journals parcel post. Within thirty days after receipt, bound volumes will be shipped prepaid anywhere in the U.S.A. Full remittance must accompany order.

The Book Shop Bindery

5811 West Division St. Chicago 51, Ill.

We are interested in studying unusual blood serums and cells for research purposes. Send your problems in blood typing, crossmatching, transfusion reactions, pregnancy, etc., to the Knickerbocker Foundation Inc., 300 West 43rd Street, New York City, and a free, complete report will be returned to you.

OFFICIAL EMBLEM

of the A. S. M. T.

Now Available in

PINS AND LAPEL BUTTONS

Supplied exclusively to members of the A.S.M.T.

Send all communications to Executive Office for approval.

This distinctive emblem is made of genuine gold or gold finish with blue enamel as a background and with pearls on the corners. The 1" size is available as a pin. The $1\frac{1}{16}$" size is available as an emblem, button, or charm at the same price.

Large Size: 10K gold $20.00; gold filled $12.50
Small Size: 10K gold $12.50; gold filled $7.50

Make check payable to Jordan Marsh Co., Smith Patterson Jewelry Division, and send with

ORDER BLANK

to

AMERICAN SOCIETY OF MEDICAL TECHNOLOGISTS
Suite 25, Hermann Professional Bldg.
Houston 25, Texas

Please Register and Place my order for

Item_____

10K Gold_____ Gold Filled_____

Name_____

Address_____

City_____

State_____

_____ Reg. No. (A.S.C.P.)

Member of the ASMT?_____

Approved:_____
Executive Secretary

DESIGNED AND MADE EXCLUSIVELY FOR THE ASMT
by Smith Patterson Co., Boston

QUALITY

KNICKERBOCKER

300 WEST 43RD STREET, NEW YORK CITY

DANGER!
CONTAINS
HEPATITIS
VIRUS

"The majority of cases of hepatitis resulted from handling clinical specimens, particularly blood specimens."

Sulkin, S. E. and Pike, R. M.—Survey of Laboratory-Acquired Infections. American Journal of Public Health—July, 1951.

Viral diseases have no respect for titles . . . the highest incidence of lab-acquired infections is among trained personnel. *Protect your people by using labels they don't have to lick with their tongues*—and don't forget —Pre-printed Time Labels can increase efficiency, cut down errors, save time in every hospital department . . . they stick to glass, wax, any container—even in Autoclaves . . . deliver accurate information—write on them with pen or pencil.

NAME_____

SPECIMEN_____

Rm. No.____ Date_____

Write for free samples and literature

SELF STICK

TIME SPECIMEN COLLECTION LABELS

Professional Tape Co. Inc. Box 41-S Riverside, Illinois

B-B-L
SENSI-DISCS

Antimicrobial Discs

50 discs per vial

for the

Reliable, Convenient and Rapid

Determination of Microbial Sensitivity

to

Antibiotics, Sulfonamides

and other

Antimicrobial Agents

Write for Folder SD-08

BALTIMORE BIOLOGICAL LABORATORY, INC.

A Division of Becton, Dickinson & Co.
Baltimore 18, Maryland

Dependency . . . or Failure

No one factor has a more important role in the achievement of conclusive results in laboratory research than the reagent used. Carworth Laboratories believes that the laboratory reagents it produces will give results equal to or more satisfactory than any others you may use.

We offer the continually increasing demand for these products as evidence of this fact.

"VACSEAL" Guinea Pig Complement
3cc 7cc 20cc

Anti-sheep HEMOLYSIN (with glycerin)
2cc 5cc 10cc

Guinea Pig KIDNEY ANTIGEN
2cc 5cc 10cc

Sheep Cells (in citrate or mod. Alsever's)
15cc 30cc 60cc (or larger)

Sterile Citrated Rabbit Blood
15cc 30cc 60cc

For further information please write to:

CARWORTH LABORATORIES, Inc.
New City, Rockland County, New York

Please Mention Publication When Writing Advertisers

You can **simplify routine urinalysis**

with

Sugar Test-**DENCO**®
(Galatest)

Acetone Test-**DENCO**

DENCO spot tests require no test tubes, no measuring, no boiling. Just a little powder... a drop of urine... that's all! You get immediate color reactions if sugar or acetone are present. *And you save time!*

Remember, if you want

SPEED... Averages 30 seconds per test

ACCURACY... No false positives

ECONOMY... Costs about 1¢ per test

then use

SUGAR TEST-DENCO (Galatest)· **ACETONE TEST-DENCO**

Both reagents are ideally suited for office use, laboratory, bedside and mass testing. And diabetics can be quickly taught to use them.

These simple tests may also be used for the detection of sugar and acetone in blood plasma.

Professional samples of both products, as well as bibliography and other descriptive material, will be mailed to you gladly upon request. Write to Dept. 155.

THE DENVER CHEMICAL MFG. CO., INC.
163 Varick Street, New York 13, N.Y.

DENCO URINALYSIS KIT – Your patients will welcome this attractive, handy kit, which fits in pocket or purse. Complete with vials of Sugar Test-Denco, Acetone Test-Denco, dropper, instructions and color chart. And you'll find it convenient, too!

Diagnostic Procedures for Virus and Rickettsial Diseases—Second Edition

This authoritative volume, first published in 1948, has been completely revised under the joint editorship of Thomas Francis, Jr., M.D., and Joseph E. Smadel, M.D. Each chapter has been completely rewritten. There is a new chapter on general principles, directed primarily to those laboratory people unfamiliar with virology and rickettsiology. In this chapter will be found a discussion of general considerations bearing directly on applied technics and a correlation of methods and technics. New chapters are also presented on tissue culture methods, Echo viruses and on the Coxsackie viruses. The volume is illustrated with 36 figures and contains 45 tables. Total pages 50 per cent greater than in First Edition.

Chapters and authors of the Second Edition are:

General Principles Underlying Laboratory Diagnosis of Viral and Rickettsial Infections. Edwin H. Lennette, M.D., Ph.D.

Poliomyelitis. John R. Paul, M.D., and Joseph L. Melnick, Ph.D.

Tissue Culture Methods for the Cultivation of Poliomyelitis and Other Viruses. Joseph L. Melnick, Ph.D.

The Coxsackie Viruses. Gilbert Dalldorf, M.D., and Grace M. Sickles.

Encephalitis (Arthropod-Borne Virus Encephalitides and Lymphocytic Choriomeningitis). W. McD. Hammon, M.D.

Rabies. Harald N. Johnson, M.D.

Influenza. Keith E. Jensen, Ph.D.

Primary Atypical Pneumonia. A. E. Feller, M.D., and Maurice R. Hilleman, Ph.D.

Mumps. John F. Enders, Ph.D., and Karl Habel, M.D.

Herpes simplex. T. F. McNair Scott, M.D.

Variola and Vaccinia. C. Henry Kempe, M.D.

Yellow Fever. John C. Bugher, M.D.

Dengue. Albert B. Sabin, M.D.

Phlebotomus (Pappataci or Sandfly) Fever. Albert B. Sabin, M.D.

Psittacosis. K. F. Meyer, M.D., and B. Eddie, Dr.P.H.

Trachoma. Phillips Thygeson, M.D.

Inclusion Blennorrhea. Phillips Thygeson, M.D.

Lymphogranuloma Venereum. Geoffrey Rake, M.B.

Miscellaneous Virus Diseases. Maurice R. Hilleman, Ph.D.

Rickettsial Diseases. Joseph E. Smadel, M.D.

Thomas Francis, Jr., M.D., and Joseph E. Smadel, M.D., Editors

596 pages 1956 $7.50

AMERICAN PUBLIC HEALTH ASSOCIATION, INC.

1790 Broadway **New York 19, N. Y.**

ALFRED E. RILEY
Medical Employment Service
Mallers Building, Room 2011A
59 East Madison Street Chicago 2, Illinois
An organization offering personal and individualized employment counseling and placement service. Conscientious and discriminating attention is given to all individuals, hospitals, and clinics served by our organization. You can negotiate confidentially with confidence. Positions are available on all levels from beginners to executives for: **Medical Technologists, Registered (ASCP) Chemist, Bio-Chemist, all levels, Bacteriologists all levels, Blood Bank Technologists and Pathologists.** Write us today regarding these interesting positions. Our negotiations are ethical and you will appreciate our personal service.
DOROTHEA BOWLBY, COUNSELOR

WANTED

MT (ASCP). 600-bed hospital. Laboratories fully departmentalized. 42-hour week. No night call. Salary open. Write to W. G. Brown, M.D., Director of Laboratories, Hermann Hospital, Houston 25, Texas.

PREGNANCY TESTS:

Male Frogs, dozen $3.50
Lots of 24 or more, per dozen $3.00
Mealworms (food), 1000 ..... $3.30

(Postage Extra on Frogs)

Free On Request: Current Live Materials List Plus Scientific Supply Catalog

Quivira Specialties Co.

4204 W. 21st St. Topeka 32, Kansas

Well-equipped 300-bed hospital affiliated with Texas Medical Center, Houston, needs MEDICAL TECHNOLOGISTS (ASCP). Excellent working conditions and liberal employee benefits such as paid vacations, group medical and hospital insurance, and an attractive retirement savings program plus 5-day work week. Available rooms and apartments suitable for family or single persons, for rent in hospital vicinity. Write, giving full particulars of your previous experience, education and training to

Personnel,
METHODIST HOSPITAL
Texas Medical Center
6516 Bertner Drive
Houston 25, Texas

HAVE YOU CHANGED YOUR ADDRESS LATELY?

PLEASE SEND US YOUR ADDRESS CHANGE so that you will receive your journal regularly. If you are going on a vacation "between journals" do NOT notify us. An address change authorized by YOU is the most satisfactory. Failure to let us know where you are located may be the reason you miss an occasional issue of the journal. (Type or block print)

Miss
Mrs.
Mr.
Sister_____
(If new, include **maiden** name)

_____
(New) No. Street

_____
City Zone State

_____
(Old) No. Street

_____
City Zone State

Tear off and mail to
THE AMERICAN JOURNAL OF MEDICAL TECHNOLOGY
Suite 25, Hermann Professional Bldg.
Houston 25, Texas

Signed: _____

ASCP REGISTERED

MEDICAL TECHNOLOGISTS

Opportunities now in well equipped general laboratory of 300-bed medical center. New England college community located in year 'round resort area. Forty-hour week. No night call. Progressive personnel policies include laundry of uniforms, health and life insurances, social security benefits.

Write:
Director of Laboratories
Mary Hitchcock Memorial Hospital
Hanover, New Hampshire

Medical Technologists—Opportunities now in modern well equipped general laboratory of 367 bed hospital. Liberal personnel policies including three weeks vacation, two weeks paid sick leave, eight paid holidays and no night or week end work. Technologists are supervised by two certified pathologists. Salary commensurate with training and experience. Apply—Director of Laboratories, Waterbury Hospital, 64 Robbins Street, Waterbury, Connecticut.

Registered Laboratory Technologists: For 275 bed teaching hospital located on Chicago's near north side, modern laboratories, starting salary $325 per month, alternate 5 and 6 day week, merit increases, excellent employee benefits, including 3 weeks vacation, 12 days paid sick leave, laundry furnished and a 50% tuition reduction on courses at Northwestern University. Apply Personnel Relations Department, Passavant Memorial Hospital, 303 E. Superior, Chicago 11, Illinois.

Memorial Hospital at Easton, Maryland, on Maryland's beautiful Eastern Shore has an opening for a laboratory technologist. Must be MT (ASCP). Starting salary $315.00 month. 44-hour week arranged to allow periodic long weekends off. Eighteen days paid vacation annually. Annual merit salary increases up to 3 years. Blue Cross group. Call or write E. C. H. Schmidt, M.D., Director of Laboratories, Memorial Hospital, Easton, Maryland, for personal interview, your expenses will be paid.

Clinical Laboratory Technologists ASCP registered or eligible, to staff laboratory which is expanding facilities. Liberal vacation, sick leave, no call. Salary depends upon qualifications. 400 bed hospital. Contact PERSONNEL OFFICE, Iowa Methodist Hospital, Des Moines, Iowa.

"Veterans" Home and Hospital Medical Technicians (Electroencephalography) $3120-$4560. Laboratory Technologists, plus Civil Service Benefits. $2940-$3900. Write: Personnel Officer, State of Connecticut, Veterans Home and Hospital, Rocky Hill, Connecticut, or the State Personnel Director, State Office Building, Hartford, Connecticut.

Texas Children's Hospital affiliated with the Texas Medical Center in Houston, Texas, has an opening for a Registered Medical Technologist (ASCP). A completely modern laboratory where routine, special and research procedures are conducted exclusively in Pediatrics. Good starting salary, excellent working environment, liberal employee benefits. Call or write Personnel Dept., Texas Children's Hospital, 6021 Fannin, Houston, Texas, Phone JA 9-4451.

Qualified Medical Technologist for Hospital Laboratory; 40 hour week; no night or week-end call; two weeks paid vacation; to $360.00 per month for registered technologists; meals on duty; living quarters available; pleasant, convenient Chicago suburb. Contact Pathologist, Ingalls Memorial Hospital; EDison 3-2300, Harvey, Illinois.

Registered Medical Technologist for small, modern, well-equipped general hospital in beautiful suburb near Chicago. Laboratory under Pathologist supervision with three technicians employed. Colleges nearby. Excellent living accommodations and personnel policies. Good salary. Apply, Superintendent, Lake Forest Hospital, Lake Forest, Illinois.

Wanted At Once
Registered Medical Technologist
(ASCP)

for new modern hospital affiliated with Texas Medical Center, Houston, Texas. Good salary, commensurate with training and experience. Ample opportunity for advancement. Call or write Dan M. Queen, M.D., Director of Laboratories, St. Luke's Episcopal Hospital, 6720 Bertner Drive, Houston 25, Texas. Telephone: JA 9-4451.

American Society of Medical Technologists
AUTOMOBILE EMBLEM

½ ACTUAL SIZE

Supplied exclusively to members of the A.S.M.T.

Send all communications to Executive Office for approval and registration.

ORDER BLANK

American Society of Medical Technologists
#25 Hermann Professional Bldg., Houston 25, Texas

Please register and place my order for.....Emblems

Name.....

Address.....

City.....

State.....**Reg. No. (A.S.C.P.)**.....

Are you a Member of The American Society of Medical Technologists?.....

Plate will not carry your registry number (A.S.C.P.) but will carry the registration number of our files, showing to whom each plate is issued.

PRICE $1.75 POSTPAID

Check must accompany this order.

Approved..... Emblem No.....

Make payable to American Society of Medical Technologists, #25 Hermann Professional Bldg., Houston 25, Texas.

The World's Most Comprehensive Medical Abstracting Service

If you are not already familiar with the far-flung medical abstracting services provided exclusively by Excerpta Medica, write today for a free brochure describing the contents of the 17 classified sections which are being published every month in 1956.

More than 60,000 abstracts will be supplied by Excerpta Medica this year. More than 2,000 medical journals in fifty-seven languages will be tapped for information. Hundreds of clinical procedures and new viewpoints will be presented that have never been available to American physicians and research workers. Each section concentrates upon one major medical specialty.

Address your request to:

EXCERPTA MEDICA FOUNDATION
2 East 103d Street
New York 29, New York

Medical Technologists for modern, well equipped laboratories in 500 bed teaching hospital in America's Most Interesting City. Approved School of Medical Technology. Forty hour week, liberal employee benefits. Starting salary based on education, experience. Apply Personnel Director, Southern Baptist Hospital, New Orleans, Louisiana.

Wanted: ASCP Registered Technologists. Laboratory expansion program under way; 140 bed hospital serving community of 60,000 including the University of Connecticut. Salary open; 40 hour week plus extra for call time; 2 week sick leave; 3 week vacation; Social Security; paid holidays. Quarters available. Full time Pathologist supervision. Forty-five minutes from Hartford; cultural and educational opportunities nearby. Write Director of Laboratories, Windham Community Memorial Hospital, Willimantic, Connecticut.

Medical Technologists—Excellent positions available in Hematology research, Blood Bank, and Clinical Hematology Laboratory. 500 bed teaching hospital in West Side Medical Center. Modern Laboratory equipment; three weeks vacation, and other liberal benefits. Housing available. Apply: Personnel Office, Presbyterian Hospital, 1753 West Congress St., Chicago 12, Illinois.

Wanted Tissue Technologist: To head department in a large Gulf Coast private hospital, 350 beds, busy service, plus active outside work. Must have 5 to 7 years experience, able to train assistants, and teach in accredited school. Air conditioned laboratory; new hospital; 4 pathologists. Appointment provisionally only. Starting salary $350.00 a month plus benefits. Write AJMT—Box 6-G.

Laboratory technologist for 65 bed general hospital, located in Mass. Berkshire Hills, a year round vacation land, summer and winter sports. High beginning salary with progressive increases, one month vacation, two weeks sick leave, seven paid holidays. Contact W. B. Plunkett Memorial Hospital, Adams, Massachusetts.

MEDICAL TECHNOLOGISTS for 600 bed near North side hospital with Northwestern University affiliation. 40-hr. wk. Day hrs. Excellent salary depending on education and experience. Apply Chicago Wesley Memorial Hospital, Personnel Service Department, 250 East Superior St., Chicago 11, Illinois.

Medical Technogolist for 200 bed hospital in year round vacation area close to Tanglewood and other music festivals. Eleven technologists. 40-hour 5-day week. Salary open. Apply: Dr. W. Beautyman, Pittsfield General Hospital, Pittsfield, Mass.

Wanted: Registered Medical Technologist (ASCP) for new hospital laboratory to open soon. College degree and hospital internship desirous. Wonderful opportunity, many job benefits. Salary open. 500 bed institution affiliated with medical research unit and school of nursing. Apply to Dr. Bernard Steinberg, Pathologist, Toledo Hospital, Toledo, Ohio.

Medical Technogolist to work in Medical Center Hospital. Located in beautiful college town and ski resort on Lake Champlain. No evening or weekend hours. For further information write to Personnel Director, Mary Fletcher Hospital, Burlington, Vermont.

Histology Technician position available immediately for experienced responsible and mature individual. General hospital laboratory, southwest. Minimum starting salary $325/mo., 40 hour week, usual vacation and sick leave benefits. Give all details including references in first letter. Contact: S. B. Silverman, M.D., Diagnostic Laboratory, 1130 E. Mc Dowell Road, Phoenix, Arizona.

Certified Laboratory Technologists for 300 bed modern hospital. Maintenance available. Liberal wage and extra benefits. Write Dr. James Ramsey, Pathologist, Washington County Hospital, Hagerstown, Maryland.

Thomas ELECTRIC ROTATOR

▶ **For use in V. D. R. L., Kline, Boerner-Jones-Lukens, Rein-Bossak, Mazzini and other flocculation tests.**

3623.

ELECTRIC ROTATING APPARATUS, Thomas. For use in the V.D.R.L., Kline, Boerner-Jones-Lukens, Mazzini and other flocculation tests, to eliminate the necessity for hand shaking of micro test slides, antigen flasks, etc.

Consisting of rubber-covered floating platform 13 inches square attached to four vertical springs fastened to the corners of a metal base, enclosed in a metal guard, and with an automatic cut-off timing device, speed control rheostat and motor with worm reduction gear and driving mechanism which rotates the platform in a horizontal plane so that it circles an area ¾-inch diameter to simulate hand rotation as suggested in the original Kline technique. See U. S. Public Health Service, "*Manual of Serologic Tests for Syphilis*," 1949, and Kolmer, Spaulding and Robinson, *Approved Laboratory Technic, 5th ed. (1951), p. 856.*

Range of speed, 70 to 210 r.p.m. Automatic timing device can be set for a maximum interval of 28 minutes in steps of ½ minute. Slides can be rotated safely simply by placing them on the surface of the rubber-covered platform which is supplied with four retaining bands of rubber which can be stretched around the edges. Operation at a speed of approximately 180 r.p.m. for a period of four minutes best meets the requirements of the V.D.R.L., Mazzini, Rein-Bossak, Kline, and Boerner-Jones-Lukens tests. The motion duplicates almost exactly the excursion and vigor of hand shaking.

7049-M.

3623. Electric Rotating Apparatus, Thomas, as above described, with automatic cut-off timing device, speed control rheostat with graduated dial with arbitrary scale from 0 to 100, four rubber retaining bands, cord and plug. For use on 115 volts, a.c. or d.c. **85.50**

10% discount in lots of 12; 15% discount in lots of 36.

7049-M. Micro Test Slide, Boerner, of glass, 108 x 57 mm, with ten mould-pressed cells 15 mm diameter x 2 mm deep, each surrounded by a moat. Construction permits thorough mixing of a flattened drop of almost uniform depth. Can be rotated either by hand or on the platform of above Rotator **1.10**

10% discount in lots of 12; 20% discount in lots of 72; 25% discount in lots of 288.

More detailed information sent upon request.

ARTHUR H. THOMAS COMPANY

More and more laboratories rely on Thomas / *Laboratory Apparatus and Reagents*

P. O. BOX 779 • PHILADELPHIA 5, PA.

PRE-MED

(prepared medium)

the disposable culture plate sealed in a sterile bag

9 MEDIA NOW AVAILABLE

for routine bacteriology

- Blood Agar
- Chocolate Agar
- E.M.B. Agar
- MacConkey Agar
- S S Agar
- Bismuth Sulfite Agar

for routine mycology

- Sabouraud Dextrose Agar
- Mycosel Agar
- Littman Oxgall Agar

PRE-MEDS are optically clear, scratch-free Petri dishes containing *ready-to-streak* culture media. For maximum shelf life, each PRE-MED is sealed in a sterile polyethylene bag to preserve moisture and prevent air contamination. (Bag may be resealed for prolonged incubation.)

Gone are the tedious hours of preparing glass Petri dishes for re-use. Gone are the troubles that go with mixing your own culture media. Let Hyland do it for you—with modern, controlled, mass production methods. You get perfect, uniform, sterile preparations at what cost-conscious laboratories agree is actually less than "do-it-yourself" costs.

With PRE-MED, even perishable specimens become routine to handle. Simply open a bag, streak the plate, incubate—and after reading, destroy. Enjoy PRE-MED advantages now. In units of 3 plates of any one medium (minimum order) —50¢ per plate. Lower cost in quantity.

Hyland

HYLAND LABORATORIES

4501 Colorado Boulevard, Los Angeles 39, Calif.

Please mention publication when writing advertisers

E. COLI–SALMONELLA–SHIGELLA INFECTIONS
SIMPLIFIED LABORATORY DIAGNOSIS
E. COLI SEROLOGY (Preliminary)
A Presumptive Diagnosis May be Made and Reported At This Time ①
Confirmatory Biochemical Tests If Typical Report ②
AGAR PLATES
Mac Conkey (or Eosin-methylene blue)
Stool Specimen or Rectal Swab
PLAIN AGAR SLANT CULTURES
Salmonella or Shigella or Desoxycholate citrate
Bismuth Sulfite
Brilliant Green
UREA Medium for each tube
PROTEUS
SERUM Specimen
REPEAT AT 6 TO 10 DAY INTERVALS

LEDERLE WILL SEND YOU THIS FREE WALL CHART

For a wall-size reproduction (18 x 24) of this chart, together with procedures, techniques and interpretations for the laboratory diagnosis of enteric and other febrile diseases, write:

5463

Lederle

MEDICAL TECHNOLOGY DEPT. DA-2
LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID COMPANY
PEARL RIVER, NEW YORK